JACKSON HOLE
WYOMING, USA

SEPTEMBER 19-23, 2009

9th PEACe

Conference on

Protein Expression in Animal Cells

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Jackson Hole

Wyoming

September 19th to 24th, 2009

Table of Contents

Message from the Chair

Dear Participants,

On behalf of the Organizing Committee it is my pleasure to welcome you to the 9th PEACe Conference organized by the International Society of Protein Expression in Animal Cells. It is the intention of the Society to promote conferences that allow the presentation and discussion of research in animal and insect cell technology, which includes the production of recombinant proteins, monoclonal antibodies and viral vaccines.

The present conference is the 9th of a series, which started in 1992, first as a more highly specialized conference "The Baculovirus and Insect Cell Conference" in Interlaken, Switzerland. Since then, meetings have been held at two year intervals and the topics have been expanded to include a wider scientific interest. In the 3rd conference organized in Jersey, Channel Islands the topics were expanded to include gene delivery systems for recombinant protein expression. By the 6th meeting in Mont Tremblant, Canada the organizing committee was expanded significantly, the topics expanded even further and the present meeting title Protein Expression in Animal Cells (PEACe) was introduced. The planning of future meetings was put on a firm footing following the Crete meeting by incorporating the International Society for Protein Expression (PEIS) as a non-profit making organization. For this and other aspects of meeting planning we have had the invaluable help of Bruno Begin through his company, Hospitalite Quebec. We are pleased to acknowledge and thank Bruno for his untiring efforts in organizing the conference.

Following the tradition of past conferences in the series the location, scientific program and social events have been designed to enhance scientific discussion and interaction amongst conference participants. Past attendees have always commented favorably on both the science and the ease of meeting new colleagues. The organizing committee thanks you for participating in the Conference and wishes you a pleasant and productive stay in Jackson Hole.

Tom Kost

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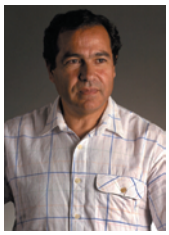

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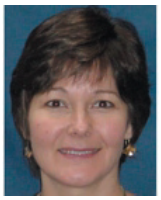

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CONFERENCE SCHEDULE 9th PEACe

Saturday September 19th 2009

18:00 - 19:00 **Welcome Reception**

19:00 - 20:00 **Opening Lecture: Kevin Taylor**, Wildlife Expeditions, Teton Science School
The Wonders of the Greater Yellowstone Ecosystem

Sunday September 20th 2009

08:15 - 08:30 **Welcome - Tom Kost**, GlaxoSmithKline
On behalf of the 9th PEACe Organizing Committee

08:30 - 10:00 **Session 1: Transient Protein Production**
Chair: Mark Smales, University of Kent

08:30 - 09:00 T1.1 Robert S. Ames, GlaxoSmithKline
The tao of BacMam

09:00 - 09:30 T1.2 Yves Durocher, Biotechnology Research Institute
Large scale transfection of CHO cells with polyethylenimine for recombinant protein production

09:30 - 10:00 T1.3 Yu-Chan Chao, Institute of Molecular Biology, Taiwan ROC
Baculovirus as a tool for gene expression in mammalian cells

10:00 - 10:30 **Coffee Break**

10:30 - 12:00 **Session 2: Stable Cells Lines & Cell Engineering**
Chair: Kim Stutzman-Engwall, Pfizer

10:30 - 11:00 T2.1 Nicholas Mermod, University of Lausanne
Iterative MAR element transfection generates highly productive cell lines

11:00 - 11:30 T2.2 Kambiz Shekdar, Chromocell
Automated genetic engineering: drug discovery platform for more physiological HTS and faster biologics production

11:30 - 12:00 T2.3 Bhaskar Thyagarajan, Invitrogen
Site-specific integrases: Applications in stem cells and screening

12:00 - 13:30 **Lunch**

13:30 - 16:00 Session 3: Protein Expression & Secretion
Chair: Kenneth Lundstrom

13:30 - 14:00 T3.1 William Bentley, University of Maryland
Tuning cells for high performance using interfering RNA

14:00 - 14:30 T3.2 Mahvash Tavassoli, King's College, London
Novel strategy for the expression and delivery of therapeutic proteins

14:30 - 15:00 Coffee Break

15:00 - 15:30 T3.3 Randal Kaufman, University of Michigan
Protein Folding, Oxidative Stress, and the Unfolded Protein Response

15:30 - 16:00 T3.4 Sean Evans, GTC Biotherapeutics, Inc.
Transgenic protein production

16:00 - 16:30 Coffee Break

16:30 - 17:30 PEACe Lecture 1: John J Rossi, Beckman Research Institute
Clinical applications of lentiviral vector mediated transduction of anti-HIV genes in hematopoietic cells for the treatment of HIV infection

18:00 - 19:00 Poster Session and Cocktail

19:00 - 21:00 Dinner

Monday September 21st 2009

08:30 - 10:00 Session 4: Post-Translational Modification
Chairs: Mike Butler, University of Manitoba and Anna Maria Moro, Instituto Butantan

08:30 - 09:00 T4.1 Michael Butler, Department of Microbiology, University of Manitoba
Strategies for enhanced productivity of a hydrophobic recombinant protein

09:00 - 09:30 T4.2 Emma Mead, University of Kent
Determination of post-transcriptional limitations on recombinant antibody production

09:30 - 10:00 T4.3 Tim Edmunds, Genzyme
Engineering glycoproteins to reduce heterogeneity and improve efficacy

10:00 - 10:30 Coffee Break

10:30 - 12:00 **Short Talks Session:** Presentations selected from submitted abstracts
Chair: Reingard Grabherr, Institut für Angewandte Mikrobiologie

10:30 - 10:45 S1. Sonja Wilke, Helmholtz Centre for Infection Research
Establishing mammalian production cell lines for structural biology by site-specific recombination

10:45 - 11:00 S2. Ela Puchacz, Millipore Corporation
Advanced Method of Cell Line Generation Using UCOE Technology

11:00 - 11:15 S3. Florian Krammer, University of Natural Resources and Applied Life Sciences
Boosting Influenza Vaccine Yield by Change of viral Morphology

11:15 - 11:30 S4. Ho Cho, Ambrx
Mammalian Expression of EuCODE Modified Therapeutic Proteins

11:30 - 11:45 S5. Barbara Maertens, Qiagen
A large scale study on the effect of human gene optimization in autologous cell-free and cellular protein expression systems

11:45 - 12:00 S6. George Brough, BD-Diagnostics, Tripath
Codon Optimized HPV E6 Proteins Overexpressed in HEK 293-EBNA cells

12:00 - 13:30 **Lunch**

13:30 - 16:00 **Workshop 1: Invitrogen**
Latest Protein Expression and Protein Gel-Electrophoresis products from Invitrogen

13:30 - 16:00 **Workshop 2: Crucell**
Advances in manufacturing of biologics using PER.C6® a human cell line

13:30 - 16:00 **Workshop 3: Invitria**
Zap-CHO Media Supplement Improves the Growth Kinetics and Productivity of CHO cells

13:30 - 16:00 **Workshop 4: Ajinomoto**
The CorynexTM Protein Expression System: a novel approach for protein production

16:00 - 17:30 **Poster Viewing**

18:00 - 21:00 **Chuckwagon Dinner**

Tuesday September 22nd 2009

08:00 **Departure to Yellowstone**

20:00 **Dinner in local restaurants**

22:00 **Back to the Hotel (on your own)**

Wednesday September 23rd 2009

08:30 - 10:30 Session 5: Alternative Cell Systems
Chair: Tom Kost, GlaxoSmithKline

08:30 - 09:00 T5.1 William L. Stanford, University of Toronto
Modeling Human Disease with Induced Pluripotent Stem (iPS) Cells

09:00 - 09:30 T5.2 Paula M. Alves. Instituto de Tecnologia Quimica, Biologica
Bioengineering long term neuronal and hepatocytes spheroid cultures for pre-clinical research

09:30 - 10:00 T5.3 Andre Choo, Bioprocessing Technology Institute, Singapore
Generation and expression of antibodies targeting cell surface antigens on human embryonic stem cells - improving the safety of stem cell therapy

10:00 - 10:30 T5.4 Stephen P Mayfield, Scripps Institute
Micro-algae as a platform for the production of therapeutic proteins

10:30 - 11:00 Coffee Break

11:00 - 12:00 PEACe Lecture 2: Michael W.Davidson, Florida State University
Recent advances in fluorescent protein technology

12:00 - 15:00 Lunch in town

15:00 - 16:30 Session 6: Large Scale Bioprocessing
Chair: Amine Kamen, Biotechnology Research Institute

15:00 - 15:30 T6.1 Sergei Zolotukin, University of Florida
An inducible system for highly efficient production of recombinant adeno-associated virus (rAAV) vectors in insect Sf9 cells

15:30 - 16:00 T6.2 Marguerite DesChamps, GlaxoSmithKline Biologicals
GSK's cervical cancer vaccine, Cervarix, manufactured with the baculovirus expression vector system

16:00 - 16:30 T6.3 Madjid Mehtali, Vivalis
Application of embryonic stem cells for the industrial manufacture of biologicals

16:30 - 17:00 Special Presentation: Verne A. Luckow, Ph.D., J.D.
Proposed Laws Regulating the Approval of Innovative and Follow-On Biologics

17:00 - 18:00 PEACe General Assembly

19:30 Gala Dinner & Entertainment

ORAL PRESENTATIONS

OPENING LECTURE

The Wonders of the Greater Yellowstone Ecosystem

Kevin Taylor
Wildlife Expeditions, Teton Science School

The Greater Yellowstone Ecosystem (GYE) is made up of Grand Teton and Yellowstone National Parks and the surrounding national forests, wildlife refuges, and other public and private lands. The Ecosystem totals approximately 18 million acres, and is considered to be the most intact ecosystem at the temperate latitudes around the world. All of the large animals that were here prior to settlement are still here, and Grand Teton National Park has been ranked in some polls to be the best place in the United States to watch wildlife. This 30-40 minutes talk (allowing 10-15 minutes for questions) will discuss the ecological and geological wonders of the GYE, and make you want to explore this wild landscape.

Session 1: Transient Protein Production

Chair: Mark Smales, University of Kent

Mammalian cell expression systems are the current gold-standard for the production of complex biopharmaceutical proteins largely due to ability of these systems to produce and secrete correctly folded and processed recombinant proteins that are functionally active. The generation of stable mammalian cell lines expressing the biotherapeutic or target of choice is a time consuming and expensive process and is a bottleneck in terms of the production of material for functional studies, assay development and screening. Further, investigations into the effects of cell engineering of particular targets on cell phenotype (e.g. growth, productivity) using stable systems limits the number of targets that can be investigated and the time required for evaluation. As a result transient gene expression is increasingly been adopted for the rapid production of recombinant proteins at the bioreactor scale and for the rapid screening of cell engineering targets. This session will discuss the latest developments in the transient gene expression field with regard to its use in these two areas of recombinant biopharmaceutical protein production.

T1.1

The tao of BacMam

Robert Ames
GlaxoSmithKline R&D, Biological Reagents & Assay Development, Collegeville, PA

Laboratories focused on drug discovery are under increasing pressure to reduce cycle times and increase screening throughput in an environment of reduced resources. Half of the high-throughput drug discovery screens are performed using cell-based assays. Cell-based assays are labor intensive and typically have long lead times for cell line generation, characterization and assay development. For every target entering the discovery pipeline there is also the need for high quality reagents for appropriate selectivity and orthologue assays. An alternative to the use of recombinant stable cell lines is to use transient gene expression to support drug discovery screening assays. We have found that the use of BacMam recombinant baculoviruses to support transient gene expression can lead to shorter cycle times for cell-based assay development and often succeeds where stable cell lines have failed. For example, in assays dependent on co-expression of multiple subunits or co-expression of multiple independent genes. For drug discovery cell-based assays BacMam technology offers numerous advantages over stable cell lines. Baculoviruses have an excellent biosafety profile and viral stocks are easily and quickly generated. Viral stocks can be prepared in weeks as compared to the months required for stable cell line generation. Gene expression levels are easily titratable and can be easily modulated. BacMam technology provides an experimental versatility simply not possible with stable cell lines. This talk will cover the principles of BacMam technology and highlight specific examples of how we have implemented it across R&D.

T1.2

Large-scale transfection of CHO cells with polyethylenimine for r-protein production

Dr Yves Durocher
Research Officer, Biotechnology Research Institute, Montreal, Canada

Large-scale transfection of HEK293 cells has proven itself as a powerful technology for the fast production of r-proteins. Many would prefer using CHO cells in order to minimize post-translational modification dissimilarities between transiently and stably expressed genes. However, CHO cells have been shown to provide lower levels of transient gene expression compared to 293 cells. We developed a simple and robust large-scale transient CHO expression platform using polyethylenimine that provides r-protein titres closely matching those obtained in 293 cells within 9 days post-transfection. The process uses the same pTT vectors that confer maximal titres in both CHO and 293 cells, making simultaneous comparison of product quality/activity and yield between the two cell lines very handy.

T1.3

Baculovirus as a tool for gene expression in mammalian cells

Catherine Y. Y. Liu, Carol P. Y. Wu, and Yu-Chan Chao
Institute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan, ROC

Baculovirus is an insect-specific virus. In insect cells, all baculovirus immediate early (IE) genes are expressed at the very early stage upon infection and instigate complex down-stream gene interactions, which is difficult to analyze. In mammalian cells, however, baculovirus genes are either not expressed or expressed at an extremely low level. Here we used a mammalian promoter to drive IE genes of baculovirus in mammalian cells, and analyze their distinct lineages of specific down-stream gene activations without the interference of other IE genes. The genes were delivered into Vero E6 cells through IE-expressing recombinant baculoviruses, and the resulting activated baculovirus transcriptome was harvested. Microarray analysis of these AcMNPV gene transcripts showed the trans-activator IE-1 significantly activated genes gp64 and pe38, and up-regulated ie-2, he65, pcna, orf-16, orf-17, and orf-25. Genes directly induced by IE-2 were unknown before, yet here we have shown both pe38 and orf-17 can be switched on by IE-2. More importantly, we discovered that the combination of IE-1 and IE-2 had a synergistic strong effect on baculovirus transcriptome, and activated around 38%, or 59 out of the 154 genes placed on the microarray. Thus, this study has provided novel revenue to delineate the complicated viral gene regulation with an un-preceded depth. We further showed that the baculovirus RING protein IE2 is a strong, promiscuous trans-activator in mammalian cells, dramatically up-regulating viral promoters such as CMV and SV40. Surprisingly, further study into the mechanism for such unique strong promoter activation leads to the discovery of a novel nuclear cage (NC)-like IE2 structure that encloses a high concentration of G-actin. IE2 mutagenesis studies indicated its ability of forming NC and trapping G-actin was crucial for IE2 activation of CMV promoter. RNA FISH and immunofluorescent experiments showed the NC is a novel active transcription center, which is visible to light microscopy. Thus, we have demonstrated a novel approach for strong stimulation of mammalian transcription, and paved a way for better application of baculovirus in mammalian protein expression and gene transfer.

Session 2: Stable Cell Lines and Cell Engineering

Chair: Kim Stutzman-Engwall, Pfizer

The development of stable mammalian cell lines is a method commonly used to produce recombinant proteins. In order to optimize stable cell line functionality, cell engineering strategies are frequently employed. This session will cover a diverse range of techniques designed to facilitate improved and/or more consistent stable cell line development.

T2.1

Iterative MAR element transfection generates highly productive cell lines

Nic Mermod
Laboratory of Molecular Biotechnology, University of Lausanne, Lausanne, Switzerland

Expression of recombinant proteins with mammalian cell lines is limited by the number of transgenes that integrate in the cell genome and by epigenetic silencing or variegation effects. We identified MAR DNA elements that mediates a specific chromatin structure and that decrease epigenetic effects when integrated in expression vectors, yielding cell clones with high and stable transgene expression.

Long-term stability studies in CHO cells indicated that one of these MARs acts to restore the expression of silent transgenes and concomitantly to protect expressed transgenes from silencing effects. Single-cell time-lapse microscopy indicated that the MAR also acts to maintain constantly elevated transgene transcription in the short term, thus preventing cells from continuously cycling between active and inactive expression. In addition to vector design improvement, an iterative transfection procedure has been devised to further increase transgene expression. It is based on multiple transfection processes that are carefully timed with relation to the cell division cycle. This yields increased genomic integration of the vector when homologous plasmids are multiply transfected. Coupled to the inclusion of MAR elements in the vector, this process yields very high transgene expression without chromosomal or expression instability.

Such vectors and processes were used to generate IgG-producing CHO cell lines with very high specific productivities. These cell lines yielded tens of grams-per-liter titers when grown in perfused bioreactors. Thus, MAR elements and optimized gene transfer methods can be used to generation high-producer stable cell lines in a short time and with little screening efforts.

T2.2

Automated cell engineering: Production of cell based assays that are accurate in vitro correlates of in vivo target activity and faster biologics production

Kambiz Shekdar
Chromocell Corporation, North Brunswick, NJ

The Chromovert drug discovery platform uses transient fluorogenic nucleic acid probes to scan millions of clones for accelerated isolation of rare, optimal cell lines even for targets that had eluded prior reduction to cell based assays. Within three months, cell based assays that represent accurate in vitro correlates of in vivo target activity "IVC" can be produced for any target regardless of its sequence or biology. Data across a diverse set of challenging targets is presented and compared to traditional production methods (e.g., addition of tags or reliance on truncated or modified receptors) and targets lacking all or accessory subunits.

Compared to previously reported cell lines which lack one or more subunits, our results for intact heteromultimeric targets such as ENaC and NaV comprising all native subunit sequences demonstrate that partial targets do not approximate the intact heteromultimeric targets as they exist in vivo. In contrast to previously published data, a panel of GABAA cell lines each expressing a different subunit combination recapitulated "gold-standard" electrophysiology in 384-well format membrane potential assays. We are using the same approach for parallel production of a combinatorial panel of cell lines for over 1,000 GABAA subunit combinations to identify fingerprints or IVCs predictive of the desired and adverse effects of known modulators. In contrast to published reports that a number of GPCRs requires addition of leader sequences for cell surface expression and function, we produced the first stable cell lines comprising corresponding native GPCR sequences. HTS resulted in compounds that were confirmed as effective in subsequent human testing and demonstrated that modification of the native receptors resulted in mutant receptors with aberrant function and pharmacology, with corresponding adverse effects on the results in HTS.

Currently, the industry average failure rate for drug discovery programs in pharmaceutical companies is above ninety percent. Although this includes failures at all stages of the process, the rate points to an urgent need for any improvements in the process. One factor contributing to the high failure rate is the lack of cell based assays that accurately model targets as they exist in vivo. The Chromovert drug discovery platform addresses this need with the scalability for systems biology applications. The technology platform has also been used for numerous inherently stable biologics cell lines that were produced in three months, alleviating the technical hurdles for cost-effective production of bioequivalent generic biologics.

T2.3

Site-specific integrases: Applications in stem cells and screening

Bhaskar Thyagarajan
Life Technologies

A family of large serine recombinases isolated from bacteriophages has previously been shown to effectively catalyze recombination between wild-type attachment sites in mammalian cells. In addition, some of these integrases can also catalyze recombination between one wild-type attachment site and one pseudo site present in the mammalian genome. This property can be exploited for use in applications ranging from gene and cell therapy to protein production and the creation of workhorse lines for drug screening. This talk will provide details on how we have adapted the system to create a robust platform for the creation of cell lines expressing genes of interest in a predictable manner. This platform can be used in virtually any cell type, and this presentation will cover some applications in human embryonic stem cells, HEK293 cells and CHO-S cells.

Session 3: Protein Expression & Secretion

Chair: Kenneth Lundstrom

Protein expression plays a crucial role in modern biotechnology at both the basic research level and from production aspects. Many factors seriously affect the efficiency of recombinant protein expression such as protein folding, ER trafficking and secretion. In addition the session contains presentations on interfering RNA approaches for altering protein expression, protein transduction domain mediated protein delivery and transgenic protein production.

T3.1

Tuning Cells for High Performance Using Interfering RNA

William E. Bentley
University of Maryland

While traditional metabolic engineering generally relies on the augmentation of specific genes and pathways in order to increase the yield of target proteins, the advent of RNA interference (RNAi) has provided metabolic engineers with another tool for altering the host cell's biological landscape in order to achieve a specific goal. Given its broad applicability and potent specificity, RNAi has the ability to suppress genes whose function is contrary to the desired phenotype. In this study, RNAi was used to increase recombinant protein production in a variety of applications including a Trichoplusia ni derived cell line (BTI-TN-5B1-4 - High FiveTM) with the Baculovirus Expression Vector System. The specific targets investigated involved key pinchpoints in cell cycle, insulin signaling, and programmed cell death. For example, Tn-caspase-1, a protease involved in apoptosis is likely the principal effector caspase present in T ni. cells. Experiments were first conducted using in vitro synthesized dsRNA to verify silencing of Tn-capase-1 and increased protein production as a result. Subsequent experiments were conducted using a cell line stably expressing in vivo RNAi in the form of an inverted repeat that results in a hairpin upon transcription. Using this construct, Tn-caspase-1 transcript levels were decreased by 50% and caspase enzymatic activity was decreased by 90%. This cell line, designated dsTncasp-2, was found to have superior viability under low nutrient culture conditions and resulted in as much as 2 times the protein yield when compared to standard High Five cells. Other successful targets will be described in our presentation along with principles that guide the use of RNAi for enhanced protein yield. Overall, our results support the application of RNAi in metabolic engineering, specifically for enhancing protein productivity in the baculovirus expression vector system.

T3.2

Novel strategy for the expression and delivery of therapeutic proteins

Mahvash Tavassoli[1] Marcella Flinterman[1], Farzin Farzaneh[2], Nagy Habib[3], Farooq Malik[2] and Joop Gäken[2]

1.Head and Neck Oncology Group, King's College London Dental Institute, London, UK; mahvash.tavassoli@kcl.ac.uk 2.Department of Haematological Medicine, King's College London, UK 3.Department of Surgical Oncology, Imperial College London, London, UK

Viruses are very efficient vehicles for the delivery of genes into cells however they have restrictions for therapeutic use such as risk of insertional mutagenesis, size limitation of the gene insert and possible immunogenicity.

The trans-acting activator of transcription (TAT) protein transduction domain (PTD) from human immunodeficiency virus type 1 mediates the transduction of peptides and proteins in a concentration-dependent and receptor-, transporter-, and endocytosis-independent manner into target cells. The TAT-PTD has an important potential as a tool for the delivery of therapeutic agents. However, the production of TAT fusion proteins in bacteria is problematic because of protein insolubility and the absence of eukaryotic post-translational modification. In addition as a therapeutic tool the physical delivery of TAT fusion proteins such as direct injection into the tumour bed is inefficient as the recombinant protein may not reach all the cells within the tumour mass. Furthermore, therapeutic delivery of proteins in vivo can result in the degradation by proteases and rapid elimination by renal filtration. Therefore multiple applications of the fusion proteins would be necessary.

An attractive alternative, both for in vitro protein production and for in vivo applications, is the use of higher eukaryotic cells for secretion of TAT fusion proteins. However, the ubiquitous expression of furin endoprotease (PACE or SPC1) in the Golgi/endoplasmic reticulum, and the presence of furin recognition sequences within TAT-PTD, results in the cleavage and loss of the TAT-PTD domain during its secretory transition through the endoplasmic reticulum and Golgi. We have developed a synthetic TAT -PTD in which mutation of the furin recognition sequences, but retention of protein transduction activity, allows secretion of recombinant proteins, followed by successful uptake of the modified protein, by the target cells. This system was used to successfully secrete marker protein, green fluorescent protein (GFP), and apoptin, a protein with tumor-specific cytotoxicity. Detection of GFP, phosphorylation, and induction of cell death by TAT -GFP-apoptin indicated that the secreted proteins were functional in target cells. In conclusion this study reports the development of a novel delivery system with important potential for cancer therapeutics.

T3.3

Protein Folding, Oxidative Stress, and the Unfolded Protein Response

Randal J. Kaufman
Howard Hughes Medical Institute, Departments of Biological Chemistry and Internal Medicine. The University of Michigan Medical School, Ann Arbor, MI.

Protein folding in the oxidizing environment of the endoplasmic reticulum (ER) is energy dependent and is coupled with disulfide bond formation and N-linked glycosylation. Protein misfolding in the ER activates a conserved intracellular signaling pathway called the unfolded protein response (UPR). Strategies to engineer the UPR will be presented that may improve ER function and protein secretion.

The endoplasmic reticulum (ER) is a cellular compartment specialized for chaperone-assisted folding and post-translational modification of nascent polypeptides. Protein folding in the complex environment of the ER is unique because oxidizing conditions promote formation of disulfide bonds. Disulfide bond formation is coupled with production of reactive oxygen species, ie. oxidative stress. Disruption of ER homeostasis through increased biosynthetic load, alteration in the oxidizing conditions, reduction in calcium storage, energy depletion, or expression of folding incompetent proteins leads to accumulation of unfolded protein and activation of the unfolded protein response (UPR)(1). The UPR emanates from the ER through activation of three transmembrane sensors, IRE1, ATF6, and PERK. IRE1 is a protein kinase that has an endoribonuclease (RNase) activity that initiates a site-specific unconventional splicing reaction that removes a 26b intron within the XBP1 mRNA. Spliced XBP1 mRNA produces a potent basic leucine zipper (bZiP)-containing transcription factor of the ATF/CREB family that activates UPR gene transcription. ATF6 is a type II ER transmembrane protein that contains a bZiP domain in the cytosol and a stress-sensing domain in the ER lumen. Upon accumulation of unfolded proteins in the ER lumen, ATF6 transits to the Golgi compartment where it is cleaved by the proteases S1P and S2P to generate the cytosolic fragment that transits to the nucleus to activate transcription of a subset of UPR genes. Finally, activation of PERK by ER stress leads to inhibition of translation initiation through phosphorylation of eukaryotic initiation factor 2 (eIF2) on the alpha subunit. Paradoxically, there are several mRNAs that require eIF2□ phosphorylation for efficient translation, for example, ATF4 mRNA. ATF4 encodes a transcription factor required to activate genes involved in protein folding, anti-oxidative stress responses, and amino acid biosynthesis and transport. If the adaptive UPR cannot resolve the protein-folding defect, cells enter apoptosis. ER stress-induced apoptosis is primarily mediated through ATF4-mediated transcriptional induction of the C/EBP homologous protein CHOP.

Studies will be summarized that demonstrate the unique and essential features of the cellular response to ER stress and how ER stress is intimately coupled with oxidative stress. For example, glucose-regulated insulin production in pancreatic beta cells requires an intact PERK/eIF2□ subpathway to prevent oxidative stress (2). Our studies demonstrate anti-oxidants can improve protein folding in the ER and prevent cell death (3). Studies will show how mouse models can be used to dissect the roles of the UPR subpathways in different physiological and pathological conditions.

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T3.4

Transgenic Protein Production

Sean Evans, R.Ph, Ph.D.
Vice President or Process Development and Quality Control
GTC Biotherapeutics, Framingham, MA

Transgenic technology has enabled the production of ATryn®, antithrombin alfa, the worlds first approved transgenically derived therapeutic protein and the first available recombinant form of human antithrombin. The transgenic production method is the only one that has succeeded in producing commercial quantities of this protein. This unparalleled production capability is a result of utilizing a natural bioreactor, the mammary gland, to produce recombinant proteins. Transgenic goats, which carry the gene encoding human AT linked to a milk specific promoter, produce g/L quantities of the recombinant antithrombin in their milk. The gene construct is integrated stably in their genome and is passed through generations, ensuring consistent and limitless quantities of the product.

This technology is also being used by GTC and it's partners/clients to develop a variety of additional protein therapeutics for commercialization. The material metric for a single goat is 1-3 L of milk per day collected 300 days per year containing 2-8 g/L of therapeutic protein which translates to 0.6-7.2 kg source material protein/year/goat. This platform allows for scaling from clinical requirement levels (~20 goats ≈ 25 kg @ 5 g/L expression) to commercial levels (~80 goats ≈ 100kg) rapidly with modest source material infrastructure changes.

To ensure the pathogen safety of ATryn® the risks from pathogenic adventitious agents (viruses, prion, and microorganisms) were evaluated at the level of the goat, the milk and the removal and/or inactivation capacity of the downstream manufacturing process. The human antithrombin transgenic goats are part of a controlled, closed certified scrapie-free goat herd and are tested for a subset of pathogens. The goat milk containing antithrombin alfa is screened in vitro for microorganisms of concern and for adventitious viruses (known and unknown). The antithrombin alfa purification process results in less than 5 ppm of total contaminating proteins and has been shown to provide robust viral and prion removal/inactivation. Thus, the pathogen risk minimization measures and purity specifications in place for the antithrombin alfa manufacturing process assure the production of a safe final product, ATryn®.

LECTURE 1

Hematopoietic stem cell and systemic RNA based therapies for the treatment of HIV infection

John J.Rossi
Beckman Research Institute of the City of Hope
Irella and Manella Graduate School of Biological Sciences, Duarte, CA

The treatment of HIV infection is largely accomplished by the use of small drug therapy termed highly active retroviral therapy (HAART). Although remarkable strides have been made in controlling progression to AIDS by the use of HAART, there are emerging problems of drug resistant virus and toxicities associated with a lifelong use of this therapy. We have been working on a couple of new and different approaches for the treatment of HIV infection. One approach is to use gene therapy of hematopoietic stem cells (HSCs) in which antiviral small RNA encoding genes are introduced into these cell via lentiviral vector derived from HIV itself. The small RNAs include an RNA interference (RNAi) trigger in the form of a short hairpin RNA targeting the HIV tat/rev common exon, a hammerhead ribozyme that degrades the CCR5 co-receptor mRNA, and a nucleolar localizing TAR decoy that binds HIV Tat and sequesters it from the virus. This triple combination inhibits entry, but also inhibits post-entry replication events. The triple combination has been transduced into HSCs derived from HIV infected AIDS/lymphoma patients whose lymphoma can be treated by autologous stem cell transplant. We have treated four patients with this vector in a "first in man" trial. The results to date show that in three of the patients there is long term gene marking and expression of the transgenes. To date all of the patients are healthy, and some increase in expression of the transgenes has been observed in one of the patients. The approaches and results of the stem cell gene therapy will be discussed.

A second approach that we are testing involves the development of RNA aptamers that bind the HIV envelope protein gp120 with low nanomolar dissociation constants. The aptamers are effective at blocking HIV infection via binding to the viral envelope. In addition the aptamer binds to cell surface expressed gp120 in infected cells and is internalized into the cytoplasm. We have taken advantage of this property to deliver anti-viral siRNAs selectively to HIV infected cells. The aptamer-siRNA chimeras provide dual inhibitory function and potently block HIV replication in a humanized mouse model for HIV infection. Details and results from this novel approach for treatment of HIV and possible strategies for eradication of HIV reservoirs using this approach will be presented.

Session 4: Post-translational modification

Chairs: Mike Butler, University of Manitoba
and **Anna Maria Moro**, Instituto Butantan

Post-translational modifications (PTMs) influence the biological activity of glycoproteins, as well as in vivo residence time, immunogenicity and structural stability. As many natural proteins present significant molecular heterogeneity of glycan isoforms, it is important to understand the acceptable biological range for biopharmaceuticals. Apart from glycosylation other frequent PTMs include amination, phosphorylation, carboxylation, hydroxylation, sulfation, folding, and pegylation. For therapeutic glycoproteins the regulatory authorities are raising the standards for demonstrating product consistency thus making process development and in-process control of PTM essential at the industrial level. The development of sophisticated analytical tools has demonstrated the feasibility of ensuring consistent quality of products. This session will present and discuss examples of PTM and its control for product integrity of recombinant glycoproteins.

T4.1

Strategies for enhanced productivity of a hydrophobic recombinant protein

Michael Butler, Kevin Sunley, Tharmala Tharmalingam, Jose Rodriguez and Maureen Spearman
Department of Microbiology, University of Manitoba, Winnipeg, CANADA

Recombinant human beta-interferon (ß-IFN) is a naturally occurring cytokine with anti-viral and anti-proliferative properties that is used presently for therapy in the treatment of multiple sclerosis. The challenge for production of ß-IFN is that it is a hydrophobic glycoprotein that tends to aggregate in cell culture, causing significant difficulty in isolation and downstream processing. Any methods aimed to enhance production ß-IFN must take into account the need for isolating its monomeric form but without compromising its glycan profile, which has been shown to be important for clinical efficacy.

We have tested various chemical additives to serum-free cultures of CHO cells producing ß-IFN. Of these, glycerol was found to be the most valuable for stabilizing the monomeric protein. Sodium butyrate enhanced productivity but altered the glycan profile. Monomeric ß-IFN was more stable at lower temperatures which led to the development of a biphasic temperature-shift regime with controlled production under hypothermic culture conditions. These conditions were optimized by a timely shift from physiological to hypothermic conditions during the culture to maximize volumetric production and reduce product aggregation. Specific productivity was enhanced further by entrapping the CHO cells in Cytopore macroporous carriers which, when combined with the temperature-shift regime, resulted in a 9.9-fold increase in monomeric protein with minimal aggregation compared to control cultures.

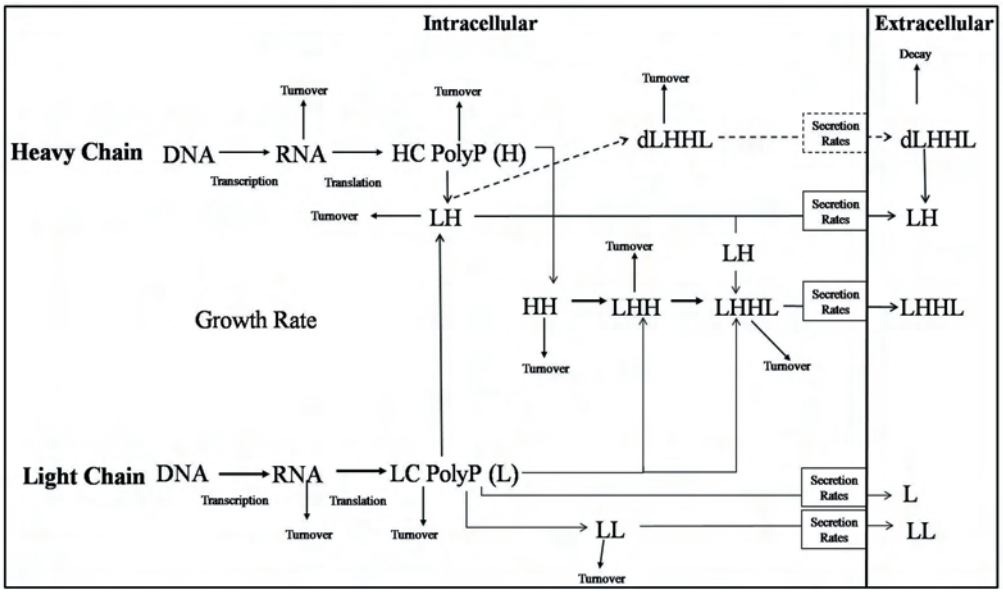
An alternative strategy enabled us to adapt CHO cells to enhanced growth under hypothermic culture conditions. The adapted cells achieved a growth rate 2-fold greater than non-adapted cells while maintaining a sustainable increase in specific productivity (QP). However, the low-temperature-adapted cells were fragile with increased sensitivity to hydrodynamic stress. The problem was resolved by using the macroporous carriers which protected the cells and allowed growth of high-density cultures under hypothermic conditions.

T4.2

Determination of Post-Transcriptional Limitations on Recombinant Antibody Production

Emma J. Mead, Lesley M. Chiverton, Tobias von der Haar and C. Mark Smales
Protein Science Group, Research School of Biosciences, The University of Kent, Canterbury, Kent

To date, the major enhancements in recombinant protein (rP) production have been achieved by advances in viable cell concentrations in the bioreactor via media development and the design of highly efficient vector systems that yield large amounts of mRNA. Despite routinely achieving relatively high levels of mRNA, this has not always resulted in higher rP expression suggesting that the major constraints are post-transcriptional. Previously we have shown, using a model rP luciferase, that expression levels are limited at a range of control points in the gene expression pathway from transcriptional to post-translational. For multi-gene, multi-domain proteins such as antibodies, gene expression can be regulated at many levels (see schematic), and thus determining the molecular mechanisms that underpin productivity is not a trivial task. Previous studies have attempted to identify constraints on isolated points in the gene expression pathway of rPs. Here, we have used parallel quantification of the abundance and stability of gene expression pathway intermediates to identify constraints on productivity. We have applied this approach to four NS0 murine myeloma cell lines with different capacities for production of a monoclonal antibody. Our results suggest that productivity is determined by limitations at a number of points throughout the gene expression pathway and each of these is considered in terms of significance with regard to rP expression from mammalian cells.



Heavy chain (H), Light Chain (L), polypeptide (PolyP), non-covalently bonded antibody (dLHHL).

T4.3

Engineering Glycoproteins to Reduce Heterogeneity and Improve Efficacy

Tim Edmunds
Genzyme Corporation

N-linked glycosylation is an important post-translational modification for many therapeutic proteins, correct glycosylation can greatly enhance the therapeutic potential of proteins through improved stability, increased half-life and improved cellular and tissue targeting. In contrast, incorrect glycosylation can lead to reduced potency due to increased hepatic clearance and/or reduced target receptor binding. Carbohydrate engineering has therefore become a critical aspect in the development of many therapeutic proteins.

In selecting an engineering strategy it is important to first understand the normal glycosylation profile of a protein and the role it plays in the biological function of that protein. In many cases glycosylation can have both positive and negative effects on biological properties. For example, increasing the level of sialylation of thyroid stimulating hormone (TSH) results in a longer circulating half-life and increased bioavailabilty but at the same time reduces binding to the TSH receptor decreasing bioactivity. In this example, isolation of the glycoprotein from plasma can give rise to misleading results regarding the desired glycoprofile since the longer circulatiing forms will be more abundant even though they may less bioactivity.

In the development of therapeutic proteins we have employed several different strategies to engineer glycosylation. These include the addition or removal of glycosylation sites through site specific mutagenesis, addition or removal or individual sugar residues with glycosidases and glycosyltransferases, engineering/selecting mutant cell lines to modify expression of glycosyltransferases, using inhibitors to metabolically modify glycosylation and chemically conjugating neoglycans to proteins. The advantages of each approach will be discussed and the rationale behind each illustrated with specific examples.

SHORT TALKS SESSION
Chair: Reingard Grabherr, Institut für Angewandte Mikrobiologie

Monday September 21st: 10:30 - 12:00

10:30 - 10:45 **S1: Sonja Wilke**, Helmholtz Centre for Infection Research
Establishing mammalian production cell lines for structural biology by site-specific recombination

10:45 - 11:00 **S2: Ela Puchacz**, Millipore Corporation
Advanced method of cell line generation using UCOE technology

11:00 - 11:15 **S3: Florian Krammer**, University of Natural Resources and Applied Life Sciences
Boosting influenza vaccine yield by change of viral morphology

11:15 - 11:30 **S4: Ho Cho**, Ambrx
Mammalian Expression of EuCODE Modified Therapeutic Proteins

11:30 - 11:45 **S5: Barbara Maertens**, Qiagen
A large scale study on the effect of human gene optimization in autologous cell-free and cellular protein expression systems

11:45 - 12:00 **S6: George Brough**, BD-Diagnostics, Tripath
Codon Optimized HPV E6 Proteins Overexpressed in HEK 293-EBNA cells

Establishing mammalian production cell lines for structural biology by site-specific recombination

Sonja Wilke[1], Sarah Torkaski[1], Volker Jäger[1], Joop van den Heuvel[1], Manfred Gossen[2], Ermanno Gherardi[3] and Konrad Büssow[1]

1. Recombinant Protein Expression (RPEX), Department of Structural Biology, Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany. 2. Max-Delbrück-Center for Molecular Medicine, 13125 Berlin, Germany. 3. MRC Centre and Laboratory of Molecular Biology, Cambridge, UK

Mammalian cell culture techniques are becoming more and more important for recombinant protein production in structural studies. In particular, crystallography requires large amounts of high-quality protein. Due to the long and expensive generation process of stable mammalian producer cell lines, new strategies involving fluorescence activated cell sorting (FACS) and recombination systems promise improvement.

In this study, different Flp mediated recombination strategies were applied on a CHO Lec cell line. CHO Lec cell lines are glycosylation deficient and their glycoprotein products can be deglycosylated and crystallized efficiently. Our master cell lines were generated with an eGFP marker gene and FACS selection of fluorescent cells. The eGFP coding region was flanked by Flp recombinase targets (FRT). We routinely obtained clonal master cell lines with high and stable GFP expression over several months. Depending on the strategy, we either exchanged GFP in the master cell line against another gene by recombinase mediated cassette exchange (RMCE) or excised eGFP by site directed recombination. In the latter case, the gene of interest was already located downstream of the GFP cassette. Upon deletion of GFP by recombination, the gene of interest was expressed. Establishing a production cell line from a master cell line by RMCE took about one month while the GFP excision method required four months.

Production cell lines for human hepatocyte growth factor (HGF) and members of the lysosomal associated membrane proteins (LAMP) family were established by GFP excision and RMCE. Cell lines established by RMCE and GFP excision showed similar productivity and compared favourably to cell lines established by a traditional approach. Protein crystals of a LAMP domain were obtained upon deglycosylation of the purified protein.

Our results indicate that the combination of FACS and site-specific recombination enables fast and reproducible cloning of protein producer cell lines that are stable without antibiotics.

Advanced Method of Cell Line Generation Using UCOE® Technology

Kim Mann, Anjali Verma, Joe Orlando, Ela Puchacz
Millipore Corporation, Bioprocess Division, Bedford, MA., USA

Isolation of highly-productive stable cell lines is the primary obstacle in the process of therapeutic protein production. Methods such as gene amplification and automated clone selection have been somewhat successful in overcoming this barrier. However, these methods have added additional problems such as increased timelines, instability of expression and increased cost to the process. New technologies that permit isolation of highly-productive cell lines in shorter times are attractive to the industry. UCOE (Ubiquitous Chromatin Opening Element) technology overcomes impediments of cell line development by producing numerous clones from a single transfection that are both highly-productive and stable. UCOE technology removes the need for gene amplification or automated clone selection and reduces the time required for cell line development to four months. UCOE elements are modeled from regions upstream of constitutively-expressed house-keeping genes and function to prevent cellular transcriptional silencing mechanisms. The process of cell line development using UCOE technology is shown. Antibody-expressing cell lines generated with and without UCOE technology are compared for protein production, transcriptional activity, stability and gene copy number.

CHO cell lines generated with UCOE elements produce 5-50 fold higher titers than control cultures and the long term stability of expression (tested for 72 days) is superior to non-UCOE controls. A detailed analysis demonstrates that the decline in the expression of few instable clones was due to the loss of transgene copy number rather than the failure of UCOE to maintain the transcriptionally active non-methylated promoter status. A CHO S culture generated with a UCOE vector producing 1.7 g/L of an IgG1 protein is shown.

These studies demonstrate that UCOE technology can help the industry overcome an important obstacle for recombinant protein production.

Boosting Influenza Vaccine Yield by Change of viral Morphology

Florian Krammer, Theresa Schinko and Reingard Grabherr
Institute of Applied Microbiology, University of Natural Resources and Applied Life Sciences, Vienna, AUSTRIA

Recent outbreaks of influenza A highlight the importance of rapid and sufficient supply for pandemic and inter-pandemic vaccines. Classical manufacturing methods for influenza vaccines fail to satisfy this demand. Alternatively, cell culture based production systems for inactivated influenza vaccines and virus-like particle (VLP) based technologies have been established. We are developing a system which can be used for enhancement of vaccine yield in both systems. By mutation of the influenza M1 gene we are able to change the influenza particle morphology from spherical to rod-shaped. These modified particles have an enhanced surface and are therefore able to carry more hemagglutinin and neuraminidase molecules per particle than standard spherical particles. Our system can be easily introduced into baculovirus-based influenza VLP production. On the other hand we have developed a baculovirus-based influenza A reversed genetics system for introduction of our modified M1 gene into influenza virus strains. Both systems can be used to produce vaccines within a few weeks. Analytics are based on methods like hemagglutination assay, ELISA, Western blot, electron microscopy and titer estimation by plaque assay.

Mammalian Expression of EuCODETM Modified Therapeutic Proteins

Barney Barnett , Frank Song, Joe Sheffer, Trung Phuong, Zinah Baray, Kyle Atkinson, Bob Holzinger, Stephanie Chu, Feng tian, Denise Krawitz, Anna-Maria Putnam, Anthony Manibusan, and Ho Cho
Ambrx, USA

Ambrx's proprietary suppression technology, (based on the phenomenon known as amber codon suppression), allows for genetic encoding of novel amino acids beyond the natural twenty into any protein, including those with therapeutic value. Our bacterial genomics based RECODETM technology enables precise chemical modification of proteins displaying improved pharmacological and biological properties. In an initial application of this technology, the novel amino acid p-acetylphenylalanine (pAF) is incorporated into human growth hormone (hGH). Strategic placement of the pAF residue provides site selective chemical attachment points for a new PEGylation (PEG) chemistry. As a result, the pharmacodynamic performance of hGH is sufficiently enhanced to create a viable long-acting therapy. Additionally, for human interferon beta (IFN'), selective molecular positioning of a pAF-PEG conjugate affords an increased in vitro potency. In this report, we summarize advances made in the expansion of the E. coli RECODETM technology to a mammalian cell expression system: EuCODETM. Chinese Hamster Ovary (CHO) cells are an effective and accepted host for expressing genes coding for proteins in which glycosylation is critical for activity, effector function, and/or pharmacology. Integrating EuCODETM technology to effect de novo incorporation of novel amino acids provides site specific loci for chemical linkages of secondary proteins, PEG molecules, active peptides, potent toxins, nucleic acids, or lipids to already highly functional glycoproteins. Application of this platform technology is demonstrated in single polypeptide glycoproteins, antibody Fc fragments, and full length humanized antibodies. Potential enhanced therapeutic implications of such applications/ technologies/approaches are just beginning to emerge. We are producing research quantities of amino acid substituted proteins transiently in CHO cell culture to support early Structure Activity Relationship (SAR) studies, purification development, and analytical method development. Larger scale material production is supported by stably transfected CHO cell lines. Application of novel expression constructs, selection strategies, and gene amplification methodologies are yielding production cell lines suitable for a commercial scale cell culture environment.

A large scale study on the effect of human gene optimization in autologous cell-free and cellular protein expression systems

Barbara Maertens[1], Anne Spriestersbach[1], Stephan Fath[2], Christine Ludwig[3], Marcus Graf[2], Ralf Wagner[2,3] and Frank Schäfer[1]

1. QIAGEN GmbH, Hilden, Germany 2. GENEART AG, Regensburg 3. University of Regensburg, Molecular Microbiology and Gene Therapy, Germany

Efficient recombinant protein production is a prerequisite for protein characterization using activity assays, interaction studies or structure determination techniques. To overcome the challenge of low yields, in silico expression optimization and de-novo gene synthesis have become powerful tools over the past years. In the meantime, it is generally accepted that heterologous protein expression such as expression of human sequences in E.coli benefits from host-specific sequence optimization. Using a multi-parameter software tool designed by GENEART optimizing for increased mRNA half-life and expression of human proteins in human cells, we predicted that sequence optimization will have a great impact also on autologous expression systems.

To test this hypothesis whether gene optimization is also a general strategy to improve autologous expression of human genes in insect and mammalian expression systems, we have started the most thorough and systematic validation study of optimized genes to date. In total, 50 genes from the NCBI Entrez database representing important protein classes such as transcription factors, membrane proteins, kinases, cytokines and ribosomal proteins were expressed in vivo in HEK293 cells and in cell-free expression systems such as insect and CHO cell-derived lysates. Expression levels of native and synthetic genes were quantified using detection methods based on fluorescence. Results for human genes optimized for expression in insect and mammalian cells demonstrated success rates for in vivo and cell-free expression of more than 95% and of 85%, respectively. Gene optimization enhanced the protein yield up to 15-fold. Our results using insect and mammalian expression systems similarly emphasize the significant impact of gene optimization on protein expression in autologous systems. Another important application is to use these optimized, ready to use expression constructs for siRNA rescue control experiments in cell culture studies.

Codon Optimized HPV E6 Proteins Overexpressed in HEK 293-EBNA cells

George H. Brough, Steven L. Knapp, Stephen G. Simkins, Karen L. Lenz, Jeffrey P. Baker and Eric P. Dixon
Becton Dickinson Diagnostic Systems-TriPath, Research Triangle Park, NC 27709

Objective: Human papilloma viruses (HPV) have been reported to have a role in several human cancers. 'High-risk' HPV (hrHPV) types 16 and 18, for example, have been associated with high-grade cervical lesions and are present in ~ 70% of cervical neoplasias worldwide. The E6 proteins of hrHPVs have been shown to interfere with the normal functions of cell proliferation and differentiation, possibly leading to malignant transformation and tumorigenesis. Because of the central importance of hrHPV E6 proteins in cervical cancer, methods to detect E6 protein from hrHPV types can aid in the identification of patients at elevated risk for cervical cancer progression. Our efforts focused on generating recombinant hrHPV E6 proteins in serum-free, suspension HEK 293-EBNA cells and their use in downstream applications such as monoclonal antibody development. Previously, such proteins have been overexpressed in prokaryotic systems for sufficient yield and purity, but they lack post-translational modifications that mammalian cells may impart to them.

Methods & Results: HPV E6 wild-type genes were cloned into expression vectors with a C-terminal or N-terminal hexahistidine epitope tag. Recombinant proteins were expressed in insect cells (T. ni) and HEK 293 EBNA cells grown in spinner flasks. Proteins were extracted using denaturing conditions and purified by nickel affinity chromatography. Expression of the native E6 protein was sufficient from T. ni cells. By contrast, expression in transiently transfected HEK 293 EBNA cells was very poor. To improve expression levels in HEK 293-EBNA cells, each gene was entirely codon optimized for the most abundant human tRNAs. This strategy, coupled with positioning of a hexahistidine tag at the N-terminus, resulted in a dramatic increase in HPV E6 expression. Both T. ni and HEK 293-EBNA expressed proteins were purified and immunized into mice using conventional and rapid approaches for generating monoclonal antibodies. Somatic fusions were performed between either splenocytes or lymphocytes and an optimized fusion partner. The resulting hybridoma cell lines were dilution cloned and the immunoglobulins were purified by protein-A affinity chromatography. Antibodies showed reactivity to both T. ni and HEK 293-EBNA expressed E6 proteins both in ELISA and Western blot. Several antibodies demonstrated cross reactivity to additional hrHPV E6 proteins.

Conclusions: Epitope tag position and codon optimization were critical factors for successful overexpression of HPV E6 proteins in HEK 293-EBNA cells. The purified recombinant proteins proved useful as immunogens for developing monoclonal antibodies. The approaches describe herein could be helpful when attempting to express other 'difficult' genes in mammalian expression systems.

Session 5: Alternative Cell Systems

Chair: Tom Kost, GlaxoSmithKline

The session will be designed to cover non-standard cell systems for the production of recombinant proteins as opposed to CHO, HEK, BHK etc. Topics covered will include the application of stem cells, 3D cell culture systems and algal cell culture for protein production. Other potential topics may include cell QC, RNAi and plant cell culture.

T5.1

Modeling Human Disease with Induced Pluripotent Stem (iPS) Cells

William L. Stanford
Institute of Biomaterials & Biomedical Engineering,
the University of Toronto, Ontario Human Induced Pluripotent Stem Cell Facility, Toronto, Ontario

Induced pluripotent stem (iPS) cells are generated by activating pluripotency transcriptional networks in somatic cells by over-expression of pluripotency transcription factors and the induction of chromatin remodeling by transcription factors or small molecules. iPS cells, like embryonic stem (ES) cells, have the capacity to make all cell types of the body and thus have potential to impact on diseases of all organ systems in future regenerative medicine applications. However, the immediate impact of iPS cells will be to study and model human monogenic and complex genetic diseases. Patient- and disease-specific iPS cell lines can be generated and induced to differentiate into the affected cell type to study the cellular basis for the disease. Our primary interest is to identify cellular phenotypes and develop high throughput screens based on these phenotypes for drug discovery. Therefore, in July 2008, we founded the Ontario Human Induced Pluripotent Stem Cell Facility to develop a biobank of somatic and reprogrammed patient iPS cell lines, which is currently has more than a dozen diseases represented – ranging from cardiac, neural, and musculoskeletal. Furthermore, the Facility is developing best practices in reprogramming technologies focused on establishing, banking and characterizing normal and patient-specific primary fibroblast cell lines and their subsequent human iPS cell derivatives. Technical advances impacting primary iPS cell induction and the quality of derivative products, such as non-viral reprogramming factor delivery, are being adapted and applied. Furthermore, the Facility is developing streamlined characterization protocols including multiplex gene expression profiling and flow cytometry-based assays to evaluate iPS cell clones. The Facility also serves an important role in providing researchers training and direct access to cutting edge iPS cell technologies for independent research endeavors. In cooperation with the Canadian Stem Cell Network, the Facility has established training workshops to disseminate iPS cell production expertise nationally and internationally.

T5.2

Bioengineering long term neuronal and hepatocytes spheroid cultures for pre-clinical research

P. M. Alves
ITQB – Instituto de Tecnologia Química e Biológica – New University of Lisbon
IBET - Instituto Biologia Experimental Tecnológica, Oeiras, Portugal

In the last decades, complex and costly animal models have been widely used in basic research on the molecular and genetic causes of several diseases, as well as in preclinical applications such as drug discovery and toxicology. This has been mainly due to the lack of functional in vitro cellular systems, as the available models, mostly two dimensional and static, presented low physiological relevance. Furthermore, the laborious and time consuming 2D-cultures are difficult to control, with severe consequences on robustness, reproducibility and scalability of these systems hampering their possible application in Cell Therapy.

Three-dimensional settings and spheroid cultures provide a cellular context closer to what actually occurs in vivo, as cells integrate external signals, including those from cellcell direct interaction, secretion/ exchange of soluble factors and/or metabolites. Our work has focused on the development of 3D-culture systems for expansion and differentiation of clinical relevant cells, namely, primary brain cells, primary hepatocytes, adult stem cells and human embryonic stem cells, exploiting the potential of bioreactor technology. Specifically, fully controlled and hydrodynamically well characterized stirred tank bioreactors are scalable and reproducible systems that enable culture homogeneity, control of culture parameters and easy sampling.

Results concerning novel culture systems will be presented and their advantages and applicability discussed.

T5.3

Generation and expression of antibodies targeting cell surface antigens on human embryonic stem cells - improving the safety of stem cell therapy

Andre Choo
Bioprocessing Technology Institute

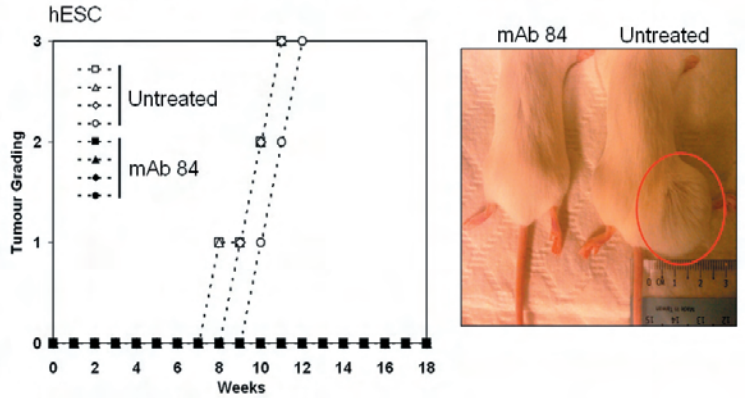
Human embryonic stem cells (hESC) can have an enormous impact on regenerative medicine because of their ability to differentiate to any cell type in the body. Despite this potential, one of the most pertinent concerns using differentiated cells from hESC is the presence of residual undifferentiated hESC, which carry a risk of teratoma formation.

Our group has recently described the generation of monoclonal antibodies (mAbs) to novel cell surface markers on human embryonic stem cells (hESC) which can be used for characterization and cell separation. Currently, hESC are characterized by a limited number of surface markers, such as stage-specific embryonic antigens (SSEA-3/4) and TRA-1-60/81. However, antibodies to these antigens were raised using human embryonal carcinomas (EC) or mouse embryos. In this study, hESC were used as immunogen for immunization. Following screening, a panel of 10 mAbs were identified which reacted strongly with undifferentiated hESC.

Remarkably, one of the clones, mAb 84, is a novel cytotoxic IgM monoclonal antibody, which selectively kills undifferentiated hESC and not differentiated cells within 30 min of incubation in vitro. The antibody binds to podocalyxin-like protein-1 (PODXL) and kills in a dose-dependent and complement-independent manner. Furthermore, in SCID mice models, we showed that teratoma formation by hESC was eliminated following treatment with mAb 84. This is compared to untreated cells which formed teratomas after 6-9 weeks post-injection (Figure 1). Mechanistically, we proposed that it binds to a stem-cell specific epitope on PODXL and triggers cell death by oncosis. More recently, we have also chimerized mAb 84 from an IgM mAb into either an IgG mAb or mAb fragments (Fab,scFv) containing human constant regions in mammalian and microbial expression systems respectively. Expression and purification of the different mAbs are currently being performed and the functional properties (binding and cytotoxicity) compared to the parental IgM mAb 84.

The significance of these findings is that this antibody can be used to eliminate contaminating hESC from the differentiated cell population prior to clinical applications because teratoma formation in vivo would severely compromise the success of the transplant. This "clean-up" step prior to transplantation will increase the safety of this procedure and alleviate concerns over the use of hESC as the starting cell population for cell therapy. To our knowledge, this is the first report of a cytotoxic mAb specifically targeting undifferentiated hESC and may prove to be an important clinical utility.

Figure 1: hESC treated with mAb 84 do not form teratomas in vivo (>24 weeks) whilst untreated cells formed after 7-9 weeks.



T5.4

Micro-algae as a platform for the production of therapeutic proteins

Stephen P. Mayfield
The Department of Cell Biology and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA, USA

The use of micro-algae as a platform for the production of protein therapeutics lags behind other organisms. This is somewhat surprising given the ability of algae to produce complex mammalian proteins in a short period of time, coupled with the ability to scale algae to very large quantities in a cost effective manner. Algae have now been shown to be capable of producing complex mammalian proteins, including monoclonal antibodies, human hormones, industrial enzymes and a variety of other potential therapeutic and vaccine molecules. Many of these proteins have also been shown to bioactive in cell based and in vitro assays. Eukaryotic algae offer tremendous potential for the large scale and cost effective production of recombinant proteins, including human and animal therapeutics

LECTURE 2

Recent Advances in Fluorescent Protein Technology

Michael Davidson
The Florida State University

Current fluorescent protein (FP) development strategies are focused on fine-tuning the photophysical properties of blue to yellow variants derived from the Aequorea victoria jellyfish green fluorescent protein (GFP) and on the development of monomeric FPs from other organisms that emit in the yellow-orange to far-red regions of the visible light spectrum. Progress toward these goals has been substantial, and near-infrared emitting FPs may loom over the horizon. The latest efforts in jellyfish variants have resulted in new and improved monomeric BFP, CFP, GFP, and YFP variants, and the relentless search for a bright, monomeric and fast-maturing red FP has yielded a host of excellent candidates, though none is yet optimal for all applications. Meanwhile, photoactivatable FPs are emerging as a powerful class of probes for intracellular dynamics and, unexpectedly, as useful tools for the development of superresolution microscopy applications.

Session 6: Large Scale Bioprocesses

Chair: Amine Kamen, Biotechnology Research Institute

This session will highlight advances and challenges in accelerating cell culture process development and scale-up for clinical and commercial manufacturing of biopharmaceuticals such as monoclonal antibodies, gene therapy vectors and vaccines. Industrial best practice examples to improve culture productivity while ensuring product quality including safety and efficiency are particularly welcome.

T6.1

An Inducible System for Highly Efficient Production of Recombinant Adeno-Associated Virus (rAAV) Vectors In Insect Sf9 Cells

George Aslanidi, Kenneth Lamb, and Sergei Zolotukhin
Division of Cellular and Molecular Therapy, Dept. Pediatrics; University of Florida

Production of clinical grade gene therapy vectors for human trials remains a major hurdle in advancing cures for a number of otherwise incurable diseases. In the current study, a novel simple and efficient system of rAAV production in insect cells is described. The system takes advantage of DNA regulatory elements from two unrelated viruses - Autographa californica multiple nuclear polyhedrosis virus (AcMNPV) and AAV2. The endpoint design consists of only two components: 1) stable Sf9-based cell line incorporating integrated copies of rep and cap genes, and 2) Bac-GOI (gene of interest flanked by AAV inverted terminal repeats). Rep and cap genes are designed to remain silent until the cell is infected with Bac-GOI helper which provides both rAAV transgene cassette and immediate-early (IE-1) transcriptional transregulator. Infection with Bac-GOI initiates the rescue/amplification of the integrated AAV helper genes resulting in dramatic induction of the expression and assembly of rAAV.

The integration cassette incorporates an IE-1 binding target sequence from wild type AcMNPV, a homologous region 2 (hr2). In addition, the cassette includes Rep-binding element (RBE), a target site for AAV2 Rep78/68 proteins. By binding to RBE, Rep proteins initiate a feed forward loop mediating the rescue/amplification of the integrated cassette. All four Rep proteins (78, 68, 52, and 40) are expressed from one uninterrupted AAV2 rep sequence thus utilizing Sf9 cells splicing machinery. Furthermore, the system had been also utilized to modulate the stoichiometry of VP1/VP2 proteins thus increasing phospholipase A2 content of the particle and improving infectivity of alternative serotypes such as AAV8 produced in insect Sf9 cells.

The arrangement provides high levels of Rep and Cap proteins in every cell thus improving rAAV yields by 10-fold. The described vectors are modular in design and could be utilized for the production of other multiprotein complexes.

T6.2

GSK's Cervical Cancer Vaccine, Cervarix®, Manufactured with the Baculovirus Expression Vector System

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Cervarix®, GlaxoSmithKline Biologicals' (GSK) cervical cancer vaccine is a recombinant vaccine composed of human papillomavirus (HPV)-16/18 L1 virus-like particles (VLP) adjuvanted with the proprietary AS04 adjuvant system. Cervarix® has been shown in phase II clinical trials to provide sustained antibody responses for HPV-16 and 18 and prevention of abnormal cytology and cervical neoplasia up to 5.5 years [Gall 2007, Harper 2007].In phase III clinical trials, the cervical cancer vaccine has been shown highly efficacious against high grade cervical neoplasia caused by HPV-16 and HPV-18 in a broad population of women and to prevent 6-month persistent infections against non-vaccine HPV types (HPV-45 and 31) [Paavonen 2007].

GSKs HPV vaccine antigens were manufactured with the baculovirus expression vector system (BEVS) using recombinant baculoviruses genetically engineered to carry the capsid HPV-16 and HPV-18 L1 gene and express the L1 proteins in a qualified cell line derived from Trichoplusia ni.

The use of the BEVS for prophylactic HPV antigen vaccine production prompted the development of a specific quality control and characterisation testing programme. Classical quality control in combination with BEVS adapted testing (e.g specific permissive cell lines and PCR) at different levels of the vaccine manufacture substantiates the quality of the BEVS for human vaccine antigen production.

Highly purified, well-characterized and immunogenic L1 VLPs are obtained following protein extraction and purification through a multistage process. The structural integrity and similarity of the L1 VLPs to the native HPV virions was documented through morphological and antigenic analysis.

Overall, quality assessment results show that the BEVS technology can be used to manufacture controlled, well-characterized, safe and scalable HPV vaccine. These results demonstrate that GSKs BEVS technology is an effective expression system for the large scale manufacturing of efficacious, safe, and immunogenic HPV recombinant vaccines.

T6.3

Embryonic stem cells for the industrial manufacture of Biologicals

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Objectives:
Primary cells (eg. chicken embryos, human fibroblasts) and established continuous cell lines (eg. MDCK, VERO, CHO) have been used since decades for the industrial production of vaccines and therapeutic proteins. However, such manufacturing systems are often limited by various drawbacks such as the risks of bacterial contaminations (regular chicken embryos), the need of large quantities of specific-pathogen free materials (eg. SPF chicken embryos), limited life span (primary cells), genetic instability (continuous cell lines), low cell densities and poor productivities. Embryonic stem (ES) cells hold exceptional biological properties that could theoretically be exploited for the derivation of new generations of cell substrates that fulfil modern industrial and regulatory requirements. Given the use since decades of avian eggs for the large scale manufacturing of human and animal vaccines, and the attractive glycosylation profile of avian species, we have generated and fully characterized stable suspension cell lines derived from duck ES cells and have demonstrated their performance in large scale production of vaccines and therapeutic proteins.

Results:
ES cells have been isolated from sanitary-controlled ducks and were used to progressively derive stable cell lines using proprietary procedures. Such cell lines, hereafter called EBx®, maintain most of the desirable features of ES cells (ie. strong expression of telomerase and stem cells surface markers, indefinite cell proliferation, long-term genetic stability...) but display new industrial- and regulatory-friendly characteristics (ie. proliferation in stainless-steel and single use bioreactors at high cell densities as suspension cells, growth in serum-free media, high susceptibility to various human and animal viruses, efficient genetic engineering and heterologous protein production...). Fedbatch processes were developed and optimized for the production of viral vaccines and monoclonal antibodies up to the 100L scale and antibodies produced on EB x® cells were shown to display a human-like glycosylation profile, with the exception of a much lower fucose content, and hence an enhanced ADCC (antibody-directed cell cytotoxicity) activity.

Conclusion:
Duck EBx® cells constitute a unique alternative for the industrial manufacture of human and veterinary vaccines as well as for the production of monoclonal antibodies with elevated ADCC activity. Fully controlled master and working cell banks are available and a Biologic Master File was filed with the US FDA. EBx® cells have already been licensed to over 25 Biotech and Pharma companies worldwide.

POSTER PRESENTATIONS

Poster Presentations

P01 A large scale study on the effect of human gene optimization in autologous cell-free and cellular protein expression systems
Barbara Maertens, Anne Spriestersbach, Stephan Fath, Christine Ludwig, Marcus Graf, Ralf Wagner and Frank Schäfer

P02 A quick and simple method for extraction and measurement of intracellular nucleotide and nucleotide sugar contents of Chinese Hamster Ovary cell lines
Georg Hinterkörner, Martin Pabst, Josie Grass, Friedrich Altmann and Nicole Borth

P03 Advanced Method of Cell Line Generation Using UCOE® Technology
Kim Mann, Anjali Verma, Joe Orlando, Ela Puchacz

P04 An unstructured kinetic model for growth and production of recombinant protein in S2 cell line
M.F. Barral, M.A. Aguiar, R.A.M. Piccoli, E.F.P. Augusto

P05 Automated high-throughput clone verification and growth analysis by Cellavista for rapid CHO cell line development
Brian Majors, Sun Ok Hwang, Shelly Martin, Gisela Chiang, Jana Dolnikova, Barbara Ehrenfels, Joanne Popoloski and Nels Pederson

P06 Autophagy in t-PA Producing CHO Cell Fed-batch Process
Mario A. Jardon, Kelsey Marshall, Beheroze Sattha, Amy O. Leung, Hélène C.F. Côté, Sharon M. Gorski, James M. Piret

P07 Case Study : An Accelerated Approach To Cell Line Development Leading To Manufacture Of Regulatory Toxicology Supplies
Timothy Gryseels, Kurt Droms, Anne Dennett

P08 Cell Culture Process Factors Affecting Monoclonal Antibody Reduction During Harvest Operations
Brian Horvath, Karthik Mani, Yael Hirsch, Kathlyn Lazzareschi, Jason Chiu, Melissa Mun, Michael W. Laird

P09 Complex Cells Supplied by Frozen Cell Supply Technology: Cell Fusion Lines, NS2 Replicon Cells, and Primary Human Lines
Sue H. Kadwell, Angela L. Rauer, Susan C. Danehower, Charlene B. McDanal, Margaret J. Gartland, Linda B. Moore, Sarah H. Gurley, Lisa A. Miller, Raymond V. Merrihew and Laurie K. Overton

P10 Design and Construction of ONE Surface Display Library for direct Screening in TWO Expression Systems
Dieter Palmberger, Gordana Wozniak-Knopp, Florian Rüker, Reingard Grabherr

P11 Developing an Efficient and Fast CHO Cell Line Development Platform
Wenlin Zeng, Cynthia Yu Kong, and Jingya Zhu

P12 Development of a cell-based HTS assay for soluble epoxide hydrolase via BacMam delivery
Wensheng Xie, Xiaoyan Tang, Quinn Lu, Robert S. Ames, Hu Li

P13 Development of a Scalable Perfusion Process for Lentiviral Vector Production by Transient Transfection
Sven Ansorge, Yves Durocher, Olivier Henry and Amine Kamen

P14 Establishing mammalian production cell lines for structural biology by site-specific recombination
Sonja Wilke, Sarah Torkaski, Volker Jäger, Joop van den Heuvel, Manfred Gossen, Ermanno Gherardi and Konrad Büssow

P15 Expression of a humanized anti-human CD18 in mammalian cells: evaluation of different strategies of whole antibody and its FvFc fragment production levels
T. M. Mello de Sousa, F. Serpieri, M. Campos da Paz, L. Ruggiero, G. M. Poças, A.M. Moro, M.M. Brígido, A.Q. Maranhão

P16 Expression of humanized constructs of an anti-CD3 monoclonal antibody in mammalian cells
F. Serpieri, C.T. de Moraes, M.S. Bezerra[2], A.Q. Maranhão, M.M. Brígido and A.M. Moro

P17 Expression of the multiprotein complex WAVE in insect cells using the MultiBac Expression System
Steffen Meyer, Robert Pluschzick, Ariane Fischer, Joop van den Heuvel

P18 FACS-Assisted Directed Evolution to obtain a CHO K1 host cell line with improved viability growing in glutamine free medium
Juan A. Hernandez Bort, Beate Stern, Nicole Borth

P19 Flow cytometry characterization of Rebmab 200
M.L. dos Santos, A.C. Pavanelli, T.L. Degaki, A.L. Inocencio, L.R. Tsuruta, L.R. Travassos, L.J. Old, G. Ritter and A.M. Moro

P20 Generation of a Full-Length cDNA Bank from Human Cell Lineages and Tissues as a basis for Human Genes Cloning and Heterologous Protein Expression
A.C.O. Carreira, L.O. Cruz, C. Colin, F.M.C. Sodré, T.M. Coelho, A.A. Camargo, M.C. Sogayar

P21 Generation of humanized Rebmab 200
T.L. Degaki, A.A. Pimenta Jr., L.R. Tsuruta, R.C. Targino, L.J. Old, G. Ritter, O.K. Okamoto and A.M. Moro

P22 High Density Insect Cell Culture for high yield manufacturing of recombinant Adeno-Associated Vectors
Jimmy A. Mena, Johnny Montes, Parminder S. Chahal and Amine A. Kamen

P23 Identification of CHO Endogenous Promoter Elements
Jens Pontiller*, Martina Baumann, Andreas Maccani, Haruthai Thaisuchat, Friedemann Hesse and Wolfgang Ernst

P24 Identification of CHO endogenous promoter elements via Chromatin Immunoprecipitation
Martina Baumann, Jens Pontiller, Haruthai Thaisuchat, Friedemann Hesse and Wolfgang Ernst

P25 Isolation, Characterization, and Cloning of Crimean-Congo Hemorrhagic Fever Virus Glycoprotein (Iran-52 strain) in a Semliki Forest Virus Vector for Mammalian Cell Expression
S.M. Ghiasi, S. Chinikar, M. Moradi, K. Lundstrom, M. Nilsson, A. Mirazimi

P26 Isolation of Chinese Hamster Ovary cell subclones with increased productivity by repeated cycles of transient transfection and fluorescence activated cell sorting
Johannes Pichler, Sybille Galosy, John Mott, Nicole Borth

P27 Lectin Binding Assays for In-Process Monitoring of Sialylation in Protein Production
Weiduan Xu, Jianmin Chen, Baisong Mei

P28 NMDA Receptor Expression in HEK293/GLAST Cells Using the BacMam Expression System
Robin T. Nelson, Nancy C. Stratman, Kathryn M. McGrath, Christine A. Strick, and Kim J. Stutzman-Engwall

P29 Optiferrin Animal-Free Recombinant Human Transferrin Supports the Growth of Cells in Serum-Free Media
Steve Pettit, Mary Ann Santos, Tanya Tanner, Joseph Camire, Paula Decaria

P30 Optimizing bioprocessing of baculovirus for gene therapy: use of metabolomic tools for production and membrane processes for purification
M.J.T. Carrondo, N. Carinhas, V. Bernal, C. Peixoto, T. Vicente, R. Oliveira, P. Alves

P31 Process Characterization and Validation of a Monoclonal Antibody Cell Culture Process Using a Quality by Design Approach
Brian Horvath, Melissa Mun, Michael W. Laird

P32 Profiling Highly-Conserved MicroRNA Expression in Chinese Hamster Ovary Cells Using Microarray and Quantitative RT-PCR
Nan Lin, Trissa Borgschulte, Katherine Achtien, Angela Davis and Kevin Kayser

P33 Protein and Membrane Supply for High Throughput Screening Assays by Transient Transfection and Baculovirus Insect Cell Expression
Georg Schmid

P34 Recombinant Histone H3.1 Provides a Non-toxic, Surface-independent and Fully-defined Reagent to Enhance Retroviral Transduction
Pascal R. Beauchesne, Michelle Miller, Katherine J. Bruce, Sanja Sekulovic, Bruce D. Bowen, R. Keith Humphries, James M. Piret

P35 Single-batch manufacturing of Recombinant Human Polyclonal Antibody drugs
Anne B. Tolstrup

P36 Stability analysis of humanized Rebmab 100 monoclonal antibody
M.L. dos Santos, A.A. Pimenta Jr., A.C. Pavanelli, M.C.B. Schmidt, A. Garbuio, M.T.A Rodrigues, L.J. Old, O. Smaletz and A.M. Moro

P37 Stability of recombinant protein production in amplified CHO cells
Zeynep Duman, Alexandra S. Croxford, Steven G. Williams, Alan J. Dickson

P38 Utilization of Site-Specific Recombination for Generating Therapeutic Protein Producing Cell Lines
Margie Campbell, Susanne Corisdeo, Clair McGee, Denny Kraichely

P01

A large scale study on the effect of human gene optimization in autologous cell-free and cellular protein expression systems

Barbara Maertens[1], Anne Spriestersbach[1], Stephan Fath[2], Christine Ludwig[3], Marcus Graf[2], Ralf Wagner[2,3] and Frank Schäfer[1]

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Efficient recombinant protein production is a prerequisite for protein characterization using activity assays, interaction studies or structure determination techniques. To overcome the challenge of low yields, in silico expression optimization and de-novo gene synthesis have become powerful tools over the past years. In the meantime, it is generally accepted that heterologous protein expression such as expression of human sequences in E.coli benefits from host-specific sequence optimization. Using a multi-parameter software tool designed by GENEART optimizing for increased mRNA half-life and expression of human proteins in human cells, we predicted that sequence optimization will have a great impact also on autologous expression systems.

To test this hypothesis whether gene optimization is also a general strategy to improve autologous expression of human genes in insect and mammalian expression systems, we have started the most thorough and systematic validation study of optimized genes to date. In total, 50 genes from the NCBI Entrez database representing important protein classes such as transcription factors, membrane proteins, kinases, cytokines and ribosomal proteins were expressed in vivo in HEK293 cells and in cell-free expression systems such as insect and CHO cell-derived lysates. Expression levels of native and synthetic genes were quantified using detection methods based on fluorescence. Results for human genes optimized for expression in insect and mammalian cells demonstrated success rates for in vivo and cell-free expression of more than 95% and of 85%, respectively. Gene optimization enhanced the protein yield up to 15-fold. Our results using insect and mammalian expression systems similarly emphasize the significant impact of gene optimization on protein expression in autologous systems. Another important application is to use these optimized, ready to use expression constructs for siRNA rescue control experiments in cell culture studies.

P02

A quick and simple method for extraction and measurement of intracellular nucleotide and nucleotide sugar contents of Chinese Hamster Ovary cell lines

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3: University of Natural Ressources and Applied Life Sciences, Departement of Biotechnology, Institute of Applied Microbiology

The development of Chinese Hamster Ovary cell lines towards higher cell densities, growth rates and productivity and better quality of the product requires a deep insight and profound understanding of the cells' metabolism, which can be provided by metabolic profiling. However, the large number of identified intracellular metabolites in mammalian cells and their variety in chemical properties still presents a problem for identification and quantification of all substances in a single step. In this study, we focused on a group of metabolites conceivably crucial for the quality of glycosylation of a recombinant protein product: nucleotides and nucleotide sugars.

A method combining solid phase extraction (SPE) and LC-MS analysis was established to identify and quantify a wide range of nucleotides and important precursors for the process of glycosylation. Our method features a relatively simple quenching step on frozen phosphate-buffered saline solution. After lysis of the pellet, nucleotides and activated sugars are recovered by solid phase extraction and eluted with a volatile alkaline buffer. Identification and quantification is done on a μ-flow LC-ESI-MS system using porous graphitized carbon (PGC) as stationary phase.

The method is fast and easy to handle. Cell lysis is simply done by addition of unbuffered sodium fluoride solution to inhibit phosphatase activity. Isotope labelled ATP is added to estimate eventual degradation or sample loss. The lysate including the internal standard can be loaded directly onto the SPE PGC cartridge without further processing. The eluate from the SPE-cartridge is then directly analyzed by mass spectrometry. We were able to detect and quantify every nucleotide sugar and nucleotide important for glycosylation including CMP-sialic acid in relatively high amounts, which can be partially destroyed using other methods like PCA extraction.

In batch cultures, intracellular nucleotide and nucleotide sugar contents of two subclones each of two different CHO cell lines producing glycoproteins were monitored with respect to their differences in metabolic characteristics and glycosylation quality.

* Georg Hinterkörner and Martin Pabst contributed equally to this work.

P03

Advanced Method of Cell Line Generation Using UCOE® Technology

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Isolation of highly-productive stable cell lines is the primary obstacle in the process of therapeutic protein production. Methods such as gene amplification and automated clone selection have been somewhat successful in overcoming this barrier. However, these methods have added additional problems such as increased timelines, instability of expression and increased cost to the process. New technologies that permit isolation of highly-productive cell lines in shorter times are attractive to the industry. UCOE (Ubiquitous Chromatin Opening Element) technology overcomes impediments of cell line development by producing numerous clones from a single transfection that are both highly-productive and stable. UCOE technology removes the need for gene amplification or automated clone selection and reduces the time required for cell line development to four months. UCOE elements are modeled from regions upstream of constitutively-expressed house-keeping genes and function to prevent cellular transcriptional silencing mechanisms. The process of cell line development using UCOE technology is shown. Antibody-expressing cell lines generated with and without UCOE technology are compared for protein production, transcriptional activity, stability and gene copy number.

CHO cell lines generated with UCOE elements produce 5-50 fold higher titers than control cultures and the long term stability of expression (tested for 72 days) is superior to non-UCOE controls. A detailed analysis demonstrates that the decline in the expression of few instable clones was due to the loss of transgene copy number rather than the failure of UCOE to maintain the transcriptionally active non-methylated promoter status. A CHO S culture generated with a UCOE vector producing 1.7 g/L of an IgG1 protein is shown.

These studies demonstrate that UCOE technology can help the industry overcome an important obstacle for recombinant protein production.

P04

An unstructured kinetic model for growth and production of recombinant protein in S2 cell line

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Drosophila melanogaster Schneider 2 (S2) cells have been used as a suitable expression system for the production of different recombinant proteins. In this study, an unstructured kinetic model was proposed to describe the growth of a transfected S2 cell line and the associated recombinant membrane rabies virus glycoprotein (RVGP) synthesis.

Cells were cultivated in a supplemented TC100 serum free medium, in a 1.5L bubble free bioreactor. Six experiments were run in batch mode, under constant dissolved oxygen tension (pO2) conditions: 5, 30, 50 or 80% (air saturation).

The proposed model formulation considers eight state variables, eighteen parameters, and the following phenomena: a) specific growth rate limited by glucose, glutamine and glutamate and inhibited by NH4+; b) preferential consumption of glutamine in relation to glutamate; c) specific death rate limited by NH4+ and inhibited by glucose and glutamine; d) specific production rates of NH4+ and RVGP partially associated to growth; e) RVGP degradation. As runs were conducted under pO2 control, their related parameters could not be fitted properly. Therefore, the model applied correction factors to represent pO2 influence on many model parameters (conversion factors and the maximum specific growth rate).The parameters were adjusted using the optimization fitting technique of flexible polyhedrons by Nelder and Mead(1) and the Gear method(2) for differential equations solutions. Model validation was done through statistical analysis (F test).

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P05

Automated high-throughput clone verification and growth analysis by Cellavista for rapid CHO cell line development

Brian Majors, Sun Ok Hwang, Shelly Martin, Gisela Chiang, Jana Dolnikova, Barbara Ehrenfels, Joanne Popoloski and Nels Pederson
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High throughput technologies in cell line development provide a way to generate high expressing cell lines with less development time in the biopharmaceutical industry. Isolating single cells enables selection for stable producing populations and consistency during subsequent scale-up as mandated by the FDA. Yet, verification of monoclonality using microscopy at the time of cell seeding in multiwell plates requires extensive time and human resources. Additionally, in an effort to shorten timelines by eliminating a subcloning step, we would like a high-throughput means to confirm monoclonality at the start of cell line development.

Therefore, we have implemented the use of the Cellavista well plate imager (Innovatis AG) which allows for image-based, non-invasive evaluation of cells in microplates. Well images obtained by brightfield illumination were analyzed for single cell verification. Photo-documentation of clonality enabled traceability of our CHO cell lines for the production of recombinant biotherapeutics and allowed for high-throughput single-cell verification following the primary transfection. In addition, during clone expansion, this instrument was used for rapid readout of cell growth in well plates. The ability to link cell number to protein concentration provides an early screen for high productivity cell lines. Here, we present our application of the Cellavista instrument for high throughput clone screening and growth analysis for the CHO cell line development.

P06

Autophagy in t-PA Producing CHO Cell Fed-batch Process

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Fed-batch protocols can result in recurrent nutrient limitations and even process failure if operated near edge-of-failure conditions. Understanding the cellular responses caused by such limitations should contribute to the rational design of robust bioprocesses. Fed-batch Chinese Hamster Ovary (CHO) cell cultures were developed in chemically defined, serum-free media, yielding 107 cells/mL and >0.25 g/L recombinant tissue plasminogen activator (t-PA). Upon amino acid depletion (particularly glutamine), apoptosis was not significantly increased and the cells continued to proliferate but with decreased productivity. However, there was evidence of increased autophagy as the lysosomal compartment increased more than 50-fold. Autophagy is a response to metabolic stress that has been far less considered than apoptosis in bioprocess-related studies. This is a degradative process by which intracellular components (proteins, cytoplasm or organelles) are delivered to lysosomes for breakdown, making available their components to compensate for medium limitations.

Chemical inhibition of autophagy with 3-methyladenine increased t-PA yields by up to 2-fold but sensitized cells to apoptosis. Inhibition of autophagic activity also correlated with an increase in mitochondrial DNA relative to nuclear DNA, an indication of increased mitochondrial content per cell. This change suggested that autophagy inhibition either imposed an energetic deficiency that upregulated mitochondrial levels, or that it prevented the clearance of damaged mitochondria in glutamine-deprived cells. Mitochondrial glutamine metabolism supplies TCA cycle intermediates required for the production of lipids and to provide reducing power for lipid and DNA synthesis. Supplementation with TCA cycle intermediates partially restored t-PA production in the absence of glutamine.

These findings indicate that the autophagic activity observed in CHO cell fed-batch cultures provides a survival mechanism that enables cells to retain a high viability upon loss of glutamine and that mitochondrial glutamine metabolism may play a key role in the interplay between apoptosis and autophagy, affecting cell proliferation, survival and recombinant protein productivity.

Keywords: autophagy, apoptosis, glutamine metabolism, fed-batch, recombinant protein production

P07

Case Study : An Accelerated Approach To Cell Line Development Leading To Manufacture Of Regulatory Toxicology Supplies

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Pfizer

In response to increasing demand to produce toxicology supplies more rapidly and reduce resource requirements for the Cell Line Development group, we employed strategies to streamline activities. Utilizing these strategies required accepting some additional risk. However, none of these risks ultimately required a re-do of any aspect of the project.

A rapid timeline was employed that ultimately produced ~225g of drug substance for toxicology studies in 9 months from the initiation of the cell line development process.

P08

Cell Culture Process Factors Affecting Monoclonal Antibody Reduction During Harvest Operations

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The potential for monoclonal antibody (MAb) disulfide reduction during cell culture harvest operations is currently a key area of focus in bioprocess development.

A cell line's susceptibility to lysis has become an increasingly important monitoring parameter as excessive cell lysis can lead to MAb reduction at the harvest step. A working hypothesis is that as cells lyse they release cellular components including macromolecules (e.g. reducing enzymes) and active proton carriers (e.g. NADPH) into the cell culture fluid (CCF). Released cellular components in turn partner to hydrolyze the interchain disulfide bonds of the MAb. Lysis susceptibility can be screened by subjecting cultures to a shear flow device that results in variable degrees of lysis between cell lines at a given energy dissipation level. The level of lysis in the CCF can be modulated by controlling the cell culture temperature during harvest operations. Factors such as cell density and viability at harvest, as well as the MAb titer also are important with respect to the overall MAb reduction risk assessment for a given process. We have found that MAb reduction kinetics in harvested cell culture fluid (HCCF) vary considerably for a single process for mixtures with varying titer and lysed cell densities. The experimental designs and results which have led to a better understanding of what influences cell lysis-mediated MAb reduction will be discussed.

P09

Complex Cells Supplied by Frozen Cell Supply Technology: Cell Fusion Lines, NS2 Replicon Cells, and Primary Human Lines

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At GlaxoSmithKline, supply of assay-ready mammalian cells as frozen aliquots has proven to save time and reagents, and assures the availability of validated fit-for-purpose cells for screening needs. It has become routine for our fermentation groups to supply frozen immortalized mammalian cell lines including HEK293 (human embryonic kidney), U-2 OS (human osteosarcoma), and CHO (Chinese hamster ovary). Protocols have also been developed for large-scale frozen cell supply of stable cell lines, cells pre-transfected with plasmid DNA, and cells pre-transduced with BacMam viruses. Recently this technology has been applied to more complex cell lines and assay formats. New developments include successful frozen cell supply for: 1) an HIV cell fusion screen, which requires pre-transduction with multiple BacMam viruses and a combination of two cell lines; 2) an HCV screen using a human liver cell line containing an HCV replicon; and 3) various screens requiring supply of human primary cell lines, with normal human lung fibroblasts and human skeletal muscle myoblasts as examples. Details for producing assay-ready frozen aliquots for these complex cell lines will include large-scale cell culture methods, harvest, aliquoting, and freezing methods. Frozen cell performance in assays will also be discussed.

P10

Design and Construction of ONE Surface Display Library for direct Screening in TWO Expression Systems

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The aim of this work was to establish an efficient method for the generation of a surface display library by using the baculoviral gene expression system displaying Fcab. Fcab molecules are antibody Fc-fragments with an antigen binding site engineered in their CH3 domains. Our strategy was to combine optimized surface display vectors with an in vitro recombination system providing sufficient library size and diversity. Viral vectors were designed to express and display Fcab on the surface of insect and mammalian cells from the same construct. Therefore the Fcab was fused to the membrane anchorage domain of Influenza A neuraminidase, controlled by a dual promoter system. Surface expression was confirmed by FACS analysis. Correct folding and dimer formation were tested with Staphylococcus aureus Protein A and human Fc□RI (CD64), respectively, which bind with high affinity to human immunoglobulin Fc regions. The functionality of the antigen binding site was investigated by binding to specific targets like "Human Epidermal growth factor Receptor 2" (Her2). For the generation of suitable libraries, an in vitro recombination system was established based on adaptation of the Gateway□ Technology by Invitrogen. Our approach demonstrated that the baculovirus surface display system is efficient for library construction and screening of antigen binding in insect and mammalian cells.

P11

Developing an Efficient and Fast CHO Cell Line Development Platform

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Development of cell lines for the manufacturing of therapeutic monoclonal antibodies is a crucial step. It typically has the most significant impact on productivity and timeline. In this presentation, we will discuss the results from our effort in establishing an efficient, fast and completely animal component free CHO cell line development process including optimization of stable cell selection parameters, selection media evaluation, and the development of a high through-put cell line screening method.

Two different approaches of generating clonal production cell lines were evaluated and compared: (1) a faster approach of generating stable cell pools and then single cell cloning and (2) directly selecting isolates in 96well plates, then single cell cloning of the top isolates. Different media were tested and found to vary significantly in their impact on selection stringency, cell productivity, and single cell cloning efficiency. An efficient high through-put FLISA method was developed for the initial screening of cell lines. His method involves no washing and reduces assay time by more than half. Also discussed will be our strategy in screening cell lines early on in cell line development process not only for productivity, but also product quality, and cell line stability to guide the selection of the right cell line and to avoid potential delay in timeline due to the need to change cell line. Data on strategies to improve productivity and reducing timeline using the GS-CHO expression system will also be presented.

P12

Development of a cell-based HTS assay for soluble epoxide hydrolase via BacMam delivery

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Epoxyeicosatrienoic acids (EETs) play important protective functions in cardiovascular and renal systems. Under physiological conditions, EETs are quickly converted to corresponding diols by soluble epoxide hydrolase (sEH) and their beneficiary effects are diminished. Inhibition of sEH therefore provides an attractive therapeutic strategy for treating cardiovascular diseases. In this report, we describe the development of a fluorescence polarization (FP) assay to quantify 14, 15-DHET in HEK293 cells overexpressing sEH via BacMam delivery, and use this assay to measure inhibition of cellular sEH activity by small molecular compounds. Upon adding exogenous EETs, sEH will convert EETs to 14, 15-DHET, which competes with Cy3B-labeled 14,15-DHET bound to anti-14,15-DHET antibody, resulting FP signal decrease. We will present data on assay development, validation and suitability of this for high throughput screening. In addition, we will compare the potency of a list of tool compounds in this assay with that of ELISA.

Key words: Soluble epoxide hydrolase (sEH), fluorescence polarization (FP), cell-based assay, high throughput screening (HTS)

P13

Development of a Scalable Perfusion Process for Lentiviral Vector Production by Transient Transfection

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Lentiviral vectors (LV) offer several advantages over other gene delivery vectors. Their potential for integration and long term expression of therapeutic genes render them an interesting tool for gene and cell therapy interventions. Significant progress has been achieved in designing safer and more efficient LV vectors. However, large scale LV production is still an important limitation for the translation of LV-based therapeutic strategies to the clinic. Therefore, the development of robust processes for mass production of LV vectors is needed. We describe here the development of a scalable process for the production of LV by transient transfection.

We exploited a suspension-grown HEK 293 cell line for the production of LV by transient PEI-based transfection with the LV-encoding plasmid constructs. Using third-generation packaging plasmids and a self-inactivating (SIN) transfer vector, we employed strategies to increase volumetric and specific productivity. The approach included the increase of cell density at the time of transfection, the comparison of several production media, the optimization of the polyplex amount per cell and the addition of the expression-enhancing additive sodium butyrate. A combination of the most promising conditions resulted in a significant increase in LV titer of more than 100-fold compared to initial conditions, reaching infectious titers of ~108 tu/mL in the culture supernatants. We successfully validated these conditions in 3 L-scale perfusion cultures and optimized the process with regards to the stability of LV under bioreactor conditions. The current transfection process is readily scalable without the need for discontinuous medium exchange. It will allow the production of LV by transient transfection in sufficiently large quantities for phase I clinical trials in 10 L bioreactor scale.

P14

Establishing mammalian production cell lines for structural biology by site-specific recombination

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Mammalian cell culture techniques are becoming more and more important for recombinant protein production in structural studies. In particular, crystallography requires large amounts of high-quality protein. Due to the long and expensive generation process of stable mammalian producer cell lines, new strategies involving fluorescence activated cell sorting (FACS) and recombination systems promise improvement.

In this study, different Flp mediated recombination strategies were applied on a CHO Lec cell line. CHO Lec cell lines are glycosylation deficient and their glycoprotein products can be deglycosylated and crystallized efficiently. Our master cell lines were generated with an eGFP marker gene and FACS selection of fluorescent cells. The eGFP coding region was flanked by Flp recombinase targets (FRT). We routinely obtained clonal master cell lines with high and stable GFP expression over several months. Depending on the strategy, we either exchanged GFP in the master cell line against another gene by recombinase mediated cassette exchange (RMCE) or excised eGFP by site directed recombination. In the latter case, the gene of interest was already located downstream of the GFP cassette. Upon deletion of GFP by recombination, the gene of interest was expressed. Establishing a production cell line from a master cell line by RMCE took about one month while the GFP excision method required four months.

Production cell lines for human hepatocyte growth factor (HGF) and members of the lysosomal associated membrane proteins (LAMP) family were established by GFP excision and RMCE. Cell lines established by RMCE and GFP excision showed similar productivity and compared favourably to cell lines established by a traditional approach. Protein crystals of a LAMP domain were obtained upon deglycosylation of the purified protein.

Our results indicate that the combination of FACS and site-specific recombination enables fast and reproducible cloning of protein producer cell lines that are stable without antibiotics.

P15

Expression of a humanized anti-human CD18 in mammalian cells: evaluation of different strategies of whole antibody and its FvFc fragment production levels

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INTRODUCTION AND OBJECTIVES: The expression of antibodies in mammalian cell cultures is still a challenge. In this work we evaluated different expression vector strategies and cell lines to express a humanized anti-CD18 antibody as a whole molecule or as fragment.

METHODS: We compared the production, by transient transfected cell cultures, with several versions of anti - CD18: 1) a quimeric FvFc (a murine scFv fused to IgG1 human CH2Ch3 domains – MUR); 2) two humanized FvFc – LL and LQ; and 3) the whole antibody (LL version) in different cell lines (CHO-K1 and BHK) evaluating two different procedures: cells transfected with one vector – H+L - harboring two expression cassettes (one for the light chain and the other for the heavy chain) cloned in opposite directions; and cells co-transfected with two different vectors (one harboring the light chain - VLC□ - and the other harboring the heavy chain – VHCH123), in different proportions (4:1, 2:1, 1:1, 1:2, and 1:4). We also obtained CHO stable clones for murine and humanized FvFc versions by co-transfection with a vector harboring the neomycin resistance gene.

RESULTS: In our study, transiently transfected CHO cells expressed the humanized FvFc versions (LL and LQ) two-fold more than the MUR FvFc version. Considering the whole antibody production, when CHO-K1 and BHK-21 cells were co-transfected with VLC□ and VHCH123, they expressed 0.89 µg/mL and 1.3 µg/mL of the humanized anti-CD18, respectively, in 1:1 proportion, which was the most efficient proportion. We observed very low production levels of anti-CD18 (less than 100□g/mL) when cells were transfected with vector H+L. From a total of 144 isolated clones for all constructions, the anti-CD18 FvFc LL producing (6) clones yielded amounts equivalent and even 3.5 fold higher than the control (non-purified human IgG at serum concentration). Just one producing clone was isolated for the LQ construction; with a 2.0 fold higher protein secretion than the control. The MUR clones showed neglected protein production.

CONCLUSIONS: Our results indicated that the strategy of co-transfection with two different vectors was more efficient for whole antibody production than the one that uses a unique vector with both chains. Among tested cell lines, the production achieved in BHK-21 was higher than in CHO-K1. Interestingly, the production of murine FvFc was lower than the humanized versions, suggesting an impairment on its expression.

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P16

Expression of humanized constructs of an anti-CD3 monoclonal antibody in mammalian cells

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Since the 90's we have been producing, at Instituto Butantan, a murine anti-CD3 monoclonal antibody (mAb) for use in transplantation rejection control. The humanization of the coding sequences of this anti-CD3 mAb was done by CDR transplantation. CHO cells were used as hosts for the expression of two constructs of hu-anti-CD3; an FvFc fragment and the whole antibody molecule. Lipofection was the method used for cells' transfection. For the expression of the FvFc fragment, the cells were selected by addition of Geneticin in the culture media; for the cloning process we used the automated equipment from Genetix (ClonePix FL, UK). Seven clones were selected for the characterization of the expressed recombinant protein by ELISA, SDS-PAGE and western blotting. Flow cytometry (Guava Technologies), using mononuclear cells from human peripheral blood, was carried out to analyze the affinity of the construct to CD3+ lymphocytes. Binding was decreased for the FvFc version as compared to the original murine anti-CD3. The construct was purified by affinity chromatography and the samples were analyzed by SEC-HPLC (Akta Purifier, GE Healthcare). The molecular mass was estimated as 100kDa. For the whole antibody expression we used the homologous recombination system Flp-In (Invitrogen). CHO cells were co-transfected with two vectors, one containing the two genes for anti-CD3 mAb and the second harboring the recombinase gene sequence. Selection was carried out by Hygromycin. For the cloning step we used ClonePix FL. Selected clones were confirmed positive by ELISA, SDS-PAGE and western blotting. Flow cytometry was used to analyze the binding of the humanized anti-CD3 to mononuclear human cells. Similar to FvFc construct, binding was lower compared to the original mAb. Difference in the binding's properties is a usual finding after the humanization process and not necessarily negative, depending on the expected effector functions for the particular monoclonal antibody.

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P17

Expression of the multiprotein complex WAVE in insect cells using the MultiBac Expression System

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The WAVE complex, a class I actin nucleation promoting factor (NPV), is crucial for the regulation of Arp2/3 activity and hence plays an important role in processes like cell motility, vesicular trafficking and pathogen infection. The exact mechanism of the interaction of the WAVE-complex with Arp2/3 and its regulation is not known until now and different models are discussed. The 3 dimensional structure of the complex will therefore be an advantageous milestone for understanding of the mode of action of the WAVE complex.

Currently, the expression of the WAVE complex is extremely difficult due to the large size of the subunits and the inherent instability of individual proteins when the complex is not correctly assembled. Expression of WAVE complex proteins for functional analyses has been shown in mammalian cells by conventional homologous recombination and in BEVS by co-infection with 5 recombinant viruses. Protein fragments of 3 out of 5 WAVE complex components could be produced in E. coli. However, these approaches are still not successful regarding high yield recombinant expression of the complete WAVE complex for crystallization and structural analyses.

Our work addresses the expression in insect cells using the Multibac expression system (in cooperation with Dr. Imre Berger, EMBL, Grenoble). In 2005 we implemented the system in our department for the expression of multiprotein complexes. This versatile tool is now used for the recombinant expression of the human WAVE-complex proteins Wave1, sra, abi, nap and HSPC300. Here we present the results of expression of the 5 protein components.

P18

FACS-Assisted Directed Evolution to obtain a CHO K1 host cell line with improved viability growing in glutamine free medium

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Introduction:
Some key points of emphasis for the generation of recombinant cell lines are cellular robustness against stress, the attainment of high cell densities as well as maintenance of high viability in order to maximise protein yield. To improve growth and viability, L-Glutamine is added as an alternative energy source due to the inefficacy of immortalised cell lines to oxidise glucose. The resulting by-product ammonium is toxic at high concentrations and has a negative impact on protein glycosylation, the major quality determining parameter of biopharmaceuticals.

Experiments and Results:
The original ATCC derived CHO K1 cell line was adapted to a chemically defined medium and suspension growth within 3 weeks. Subsequently, L-Glutamine was stepwise reduced from 8mM to 4mM and to 2mM. After each reduction, both the final cell density in batch culture and the viability were reduced. In order to force a rapid evolution of cells to continued high final cell concentration under the new conditions, cells were seeded at high densities (107/ml) and surviving cells sorted when viability dropped below 10% (typically after 24h). The sorted population was able to reach comparable or even better viable cell concentrations after this treatment and had a significantly improved viability compared to their ancestors. The 2mM glutamine adapted cell line was directly transferred into glutamine free medium and was able to continue growing without requiring further adaptation. To document maintenance of the inherent production capacity of the cell line, transient transfections of antibody plasmids were performed and shown to be unchanged.

Conclusion:
In summary, a robust cell line with high viability and high cell density was obtained by subjecting it to stress conditions similar to those encountered at the end of bioprocesses and by selecting rare cells that are exceptionally well able to survive this treatment.

P19

Flow Cytometry Characterization of Rebmab 200

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Per.C6 cells (Crucell, Netherlands) were transfected with a vector containing the genes coding for heavy and light chains of Rebmab 200 (Recepta-biopharma designation for licensed huMX35 mAb). A stable pool of the adherent growing cells was generated and the cells subsequently adapted to suspension growth in serum-free medium. The humanized antibody was purified from the cell supernatant by protein A chromatography. MAb MX35 - the original murine version of Rebmab 200 - was shown, in previous work done at LICR/NY, to bind to OVCAR-3 and a few other cancer cell lines in mixed hemadsorption assays. The target of mAb MX35 is an epitope in the external loop of the sodium-dependent phosphate transport protein 2b (NaPi2b) highly expressed on ovarian tumor cells. To verify that humanization did not alter the cell surface binding characteristics of the antibody and to screen for cancer cells expressing NaPi2b on their cell surface we tested Rebmab 200 on a panel of normal and cancer cell lines. The analysis was performed by flow cytometry using a Guava EasyCyte (Guava Technologies) cytometer. Twenty five human cell lines were tested, of which 19 were derived from tumors and 6 were considered normal human cell lines. As a negative control a commercial humanized IgG1 monoclonal antibody unrelated to cancer cells was used. We found Rebmab 200 binding selectively to two cell lines, one ovarian cell line (OVCAR-3) and one renal carcinoma cell line (SK-RC-18). Rebmab 200 antibody did not bind to a series of cancer cell lines derived from lung (non-small cell lung cancer), breast, cervix, prostate, colon, bladder, stomach and melanoma as well as a panel of normal cell lines derived from kidney, lung and retina. The observed binding characteristics were similar to the murine antibody, confirming that the humanization process utilized for the generation of Rebmab 200 did not alter the binding specificity of the antibody.

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P20

Generation of a Full-Length cDNA Bank from Human Cell Lineages and Tissues as a basis for Human Genes Cloning and Heterologous Protein Expression

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Objective: To construct a Human Full-Length cDNA Bank to facilitate successful amplification of full-length cDNAs encoding different proteins of biopharmaceutical interest for heterologous expression in animal cells and to uncover new transcripts/splicing isoforms which are expressed at low levels and/or in restricted sets of tissues.

Methods and Results: During the Human Genome Transcript Finishing Initiative (TFI), we introduced a strategy to characterize new human transcripts and splicing isoforms expressed at low levels and in restricted sets of tissues (Genome Research, 2004, 14:1413-1423). RNA from cell lines obtained from the American Type Culture Collection and from a commercial tissue panel were used in order to generate cDNAs representing distinct human tissues. The cDNAs were synthesized using Improm-II or SuperScript reverse transcriptase, characterized and validated. The cDNA quality was assessed through analysis of amplified gene fragments corresponding to four genes, namely: GAPDH, p53, NOTCH2 and MLH1. The first three genes display differential transcription: GAPDH is a housekeeping gene, with an abundant transcript, while p53 displays very low expression. The NOTCH2 transcript is very long, requiring a high-quality cDNA preparation for amplification. This allowed us to classify the cDNA quality and the integrity of the cDNA sequences which are represented in the preparations. In addition, genomic DNA contamination was verified by amplification of the human MLH1 intronic gene fragment, located in exon 12, with none of the template cDNAs used being contaminated by genomic DNA. This Human cDNA Bank allowed us to successfully amplify full-length cDNAs, encoding different proteins, particularly those with biopharmaceutical potential. As a successful example, the G-CSF and GM-CSF genes were amplified and individually subcloned into the pGEM T-Easy vector. The sequences of these cDNAs were verified by DNA sequencing and the inserts were transferred into both a mammalian cell expression vector and a baculovirus/insect cell expression vector in order to compare the expression levels in these heterologous expression systems.

Conclusion: A Human Full-Length cDNA Bank was generated and the quality of each cDNA was assessed and validated by analysis of the expression of four gene fragments: GAPDH, p53, NOTCH2 and MLH1. We successfully used this Bank to amplify full-length cDNAs encoding different proteins (cytokines, growth factors, etc.) and their isoforms, in addition to novel genes, displaying differential expression in specific tissues or cell lineages. Support: FAPESP, FINEP, CNPq, São Paulo Ludwig Institute for Cancer Research.

P21

Generation of Humanized Rebmab 200

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Rebmab 200 is a humanized version of the murine monoclonal antibody (mAb) MX35 described by LICR/NY in 1987. MX35 recognizes the sodium-dependent phosphate transport protein 2b (NaPi2b), which is expressed at high frequency in ovarian tumors and in a limited number of normal tissues. Radioisotope-labeled murine MX35 had been used in several clinical trials conducted by LICR in patients with ovarian cancer and the antibody showed good targeting to ovarian cancer including peritoneal micrometastasis. Epithelial ovarian cancer is one of the most common gynecologic malignancies with a high mortality rate. Most patients present with advanced disease because the absence of symptoms before it reaches a late stage makes it difficult to diagnose. Surgery and chemotherapy a current treatment modalities but the tumor will recur in a large percentage of patients. Targeted immunotherapies with monoclonal antibodies are explored as novel treatment modalities and the humanized antibody Rebmab 200 has been considered to be a good candidate for treatment of ovarian cancer.

Expression of Rebmab 200 is being developed in Per.C6 human cells (Crucell, Netherlands). A first pilot transfection was performed with the original adherent Per.C6 cells using lipofectin. A stable pool of transfected cells was generated by selection with G-418. Part of the pool was later re-transfected by the same procedure and mAb production in the culture supernatant was assessed by BIAcore (GE Healthcare, Sweden). For selecting clones from the transfected stable pool (adherent cells) the ClonePix FL Platform (Genetix, UK) was used. A twice concentrated culture medium was prepared and mixed with the gel matrix 1:1 to cover the monolayer of transfected cells with this semi-solid medium. Eight clones were initially selected by fluorescence using ClonePix software. Four of eight showed stable antibody production (measured by ELISA) and two clones were successfully adapted to suspension growth in serum-free medium. These two clones were then assessed for productivity in batch conditions.

Financial support: FAPESP, FINEP, CNPq and RECEPTA biopharma (Brazil).

P22

High Density Insect Cell Culture for high yield manufacturing of recombinant Adeno-Associated Vectors

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Recombinant Adeno-Associated Virus (rAAV) is one of the most promising vectors for gene therapy. Currently, rAAV vectors are being used in several clinical trials for the treatment of diseases, such as cystic fibrosis, hemophilia B, rheumatoid arthritis and Alzheimer's disease among others. Gene therapy clinical trials require large amounts of these recombinant vectors (1.0x1016 total vector genomes), but the current production technologies have several drawbacks regarding process scalability. Baculovirus Expression Vector System (BEVS) has demonstrated potential for high yield production of rAAV. Typically cell densities around 2.0x106 cells/mL are used to produce recombinant proteins, viral vectors or viral-like particles with BEVS. Operation of high cell density insect cultures for high yield production have been limited by the so-called "cell density effect", and the need of highly concentrated baculovirus stocks. The "cell density effect" is a reduction in productivity observed at cell densities higher than 2.0x106 cells/mL, and it is mainly due to high nutrient demand not supported by current medium formulations. The challenge of high density insect cell cultures is the design of a feeding strategy capable of supporting high demand for nutrients, while minimizing negative impacts on the cell productivity and osmolarity. The proposed feeding strategy consists of step-additions of a nutrient-cocktail during the cell culture, thereby avoiding any nutrient limitations during the pre-infection and post-infection stages. Cell densities up to 44.0x106cells/mL were reached in non-infected cultures. Combining the feeding strategy with coinfection at low MOI (0.1 plaque-forming units/cell), cell densities up to 10.0x106cells/mL were reached at production stage in a 3L bioreactor. Initially, this approach was evaluated in the production of rAAV serotype 2 expressing GFP as transgene. An increase of 10-fold in rAAV volumetric titer (1.17x1011 Vector Genomes/mL of cell culture) was observed, and 30 times less baculovirus stock volume was necessary in comparison with the traditional production strategy at Low cell density-High MOI (3 pfu/cell). With these encouraging results, the proposed strategy was implemented to produce different rAAV serotypes and other viral-like particles.

P23

Identification of CHO Endogenous Promoter Elements

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This approach aims to identify new CHO endogenous gene regulatory elements that are capable of controlling foreign gene expression in recombinant CHO host cells. The standard technology for the production of many biopharmaceutical products is frequently based on expression vectors that utilize strong mammalian active viral promoters like CMV or SV40 which allow for very high expression rates but this may lead to constitutive over-expression resulting in a permanent stress for the cell. In addition, some heterologous promoters are cell cycle dependent and can be subject to gene silencing generating heterogeneity within the cell population. Here we describe the construction of a genomic CHO library and the subsequent identification and isolation of selected target sequences that are believed to be responsible for high level expression of the associated genes. The method that was used to isolate these regions of interest relies on gene specific amplification with primer pairs binding on different genes and the vector sequence. Flanking regions of these fragments were identified through inverse PCR from fragmented genomic DNA. Expression levels of the derived potential promoter elements were determined through a luciferase reporter assay.

P24

Identification of CHO endogenous promoter elements via Chromatin Immunoprecipitation

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Up to 70 % of all recombinant biopharmaceuticals are produced in Chinese Hamster Ovary (CHO) cells. As endogenous regulatory elements can function consistently with the host cell mechanisms for controlling foreign gene expression in contrast to viral promoters like CMV or SV40, there is a great interest in CHO-own promoters. The great difficulty in finding such cell-own regions is that mammalian promoters are not a clearly defined unit. They show a variable structure as they are composed of a varying arrangement of short regulatory sequences. Here we describe an approach to find functional CHO endogenous promoter elements using Chromatin Immunoprecipitation (ChIP). Immunoprecipitation of the target DNA was performed using an antibody recognizing TAF1, the largest subunit of the general transcription factor TFIID, which is responsible for binding to the core promoter and recruiting the RNA polymerase II. DNA fragments isolated via ChIP were cloned into a reporter vector for further investigation of the functionality of the identified sequences. The promoter activity of the obtained regions will be proven by measurement of the expression levels of a luciferase gene in transfected CHO cells.

P25

Isolation, Characterization, and Cloning of Crimean-Congo Hemorrhagic Fever Virus Glycoprotein (Iran-52 strain) in a Semliki Forest Virus Vector for Mammalian Cell Expression

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Objective: Crimean-Congo Hemorrhagic Fever (CCHF) is a viral tick-born zoonosis disease, which is caused by the CCHF virus (CCHFV). The virus belongs to the Nairovirus genus and Bunyaviridae family, and its genome contains S, M and L segments encoding nucleocapsid, glycoprotein, and RNA-dependent RNA polymerase, respectively. The virus is transmitted to humans by infected tick bites, handling of infected blood or tissues of infected livestock or nosocomially. There is no effective vaccine against this disease. We isolated the M segment of the virus genome and cloned it into the Semliki Forest Virus vector for expression in mammalian cells and for the evaluation of the immunogenicity of the recombinant antigen.

Methods: The forward (SF) and reverse (SR) primers were designed on the basis of the CCHFV M-segment sequence in GeneBank (DQ446215). The M-segment of CCHFV was isolated by two-step RT-PCR, which required optimization of the PCR procedure. The PCR products were analyzed by restriction enzymes and also confirmed by sequencing. Sub-clonings were performed according to common genetic engineering protocols. The M-segment was cloned into the Semliki Forest Virus vector pSFV2gen and clones were confirmed by restriction enzyme analysis and sequencing.

Result: Sequence analysis of the M-segment of the CCHF virus (Iran-52 strain) revealed one major open reading frame that potentially encodes a precursor polyprotein of 1,684 amino acids in length. The mature virus glycoproteins, Gc and Gn, are generated by proteolytic cleavage from the precursor. This large M-segment introduced into the SFV vector will generate a transcript in the order of nearly 15000 nucleotides in length, which will be consequently introduced into BHK cells together SFV helper RNA for recombinant particle production.

Conclusion: It is a major challenge to isolate long CCHFV RNA genomes from CCHF patient's serum by RT-PCR. Verification of the M-segment sequence after sub-cloning into the SFV vectors will permit recombinant SFV particle generation. Recombinant SFV particles will be validated for CCHFV glycoprotein expression. Potentially SFV particles can be directly used as vaccine vectors or the recombinant CCHFV glycoprotein purified for immunization assessments and epitope mapping.

P26

Isolation of Chinese Hamster Ovary cell subclones with increased productivity by repeated cycles of transient transfection and fluorescence activated cell sorting

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Transient production of recombinant proteins in mammalian cells is a commonly used method for the rapid generation of sufficient amounts of product for initial testing. Attempts to improve yields normally target the transfection procedure, media composition or cultivation conditions. However, using an optimized protocol for transfection, we also observed significant differences in the yield obtained from different CHO host cell lines. The protocol was optimized to a degree that allowed transfection of almost 100% of the cells with reproducible productivity (sdev of 25 independent transfections: +/- 15%). Even within clonal host cell lines, the amount of intracellular antibody produced varies over a range of 10-50 fold, as determined by flow cytometry. If such variation exists within a cell population, we argued that it should be possible to isolate subclones with enhanced productivity during transient production.

For this purpose we sorted the 1% cells with the highest specific productivity from a population of CHO S cells and a proteinfree-adapted CHO K1 cell line after transient transfection. When cells were directly subcloned during sorting, no significant differences in specific production rates were observed when the expanded clones were again transiently transfected. Rather, it appears that the observed range of productivity within the population is caused by random phenotypic variation and not by stable changes in cell behaviour. On the other hand, when high producing cells were first enriched by three rounds of repeated bulk sorting of the 1% highest producers, it was then possible to isolate subclones by single cell sorting that reproducibly and stably had a three fold improved specific productivity during transient transfection. The differences between the parental cell lines and these subclones are currently being evaluated.

P27

Lectin Binding Assays for In-Process Monitoring of Sialylation in Protein Production

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Coagulation factor VIII (FVIII) is a glycoprotein which is extensively post-translationaly modified with N-linked glycosylation. The terminal sialic acid in N-linked glycans of FVIII is required for the maximal circulatory half life. Because of the therapeutic importance of the sialylation, profound commitment has been made to ensure the high level of sialylation of FVIII in manufacturing. The percent sialylation of FVIII can be determined by high pH anion-exchange chromatography coupled with a pulse electrochemical detector (HPAEC-PED) but it requires quite amount of concentrated protein through ultrafiltration and/or purification. The objective of present study is to develop assays that enable detection and prediction of sialylation deficiency of FVIII at early stage in the process, with fermenter cells or tissue culture fluid (TCF), and thus prevent downstream sialylation excursions.

Lectin ECA (Erythrina cristagalli) has highest binding affinity to Gal□1-4 GlcNAC. The level of terminal Gal□1-4 exposure indirectly reflects and is inversely proportional to the level of FVIII sialylation. By using ECA, we first developed a cell based assay to measure the global sialylation status of glycoproteins in FVIII producing cells. To examine the Gal□1-4 exposure on the FVIII molecule in fermentor TCF, we further developed an ELISA based ECA-FVIII binding assay. The ECA binding specificity in both assays was assessed by using ECA specific sugar inhibitors and neuraminidase digestion. The correlation between ECA cell surface staining and global sialylation status in fermentor cells was confirmed by ST3GAL4 RNAi knocking down experiment. To determine the correlation between Gal□1-4 exposure and the HPAEC-PED value, the fermentor TCF and UFDF samples were tested with ECA ELISA binding assay and compared with the corresponding HPAEC-PED results, an inverse correlation was exhibited.

In conclusion, cell based and ELISA based lectin ECA binding assays were developed to reveal the level of Gal□1-4 exposure, which is inversely correlated with the level of sialylation in FVIII . The assays do not require concentrated and purified material as the HPAEC-PED method does, and can be useful for tracking the status of FVIII sialylation at early fermentor stage. The assays offer great value of being cost-effective and time-saving approach for the in-process monitoring of FVIII sialylation.

P28

NMDA Receptor Expression in HEK293/GLAST Cells Using the BacMam Expression System

Robin T. Nelson, Nancy C. Stratman, Kathryn M. McGrath,
Christine A. Strick, and Kim J. Stutzman-Engwall
Pfizer Global Research & Development, USA

Expression of the N-Methyl-D-aspartate (NMDA) ion-channel receptor in non-neuronal cells is complicated by toxicity due to activation of the receptor by glutamate in cell culture media. Glutamate binds with high affinity to the NMDA receptor and as a result opens the channel which permits the influx of Ca+. The Ca+ activates various excitatory signaling pathways resulting in cell death. This can be partially overcome by the addition of the competitive NMDA antagonist (+/-)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), but not completely. In vivo, regulation of glutamate is tightly controlled by glutamate transporters. By transiently co-expressing NMDA subunits NR1a and NR2a using the BacMam expression system in a HEK293 cell line stably expressing the glutamate transporter EAAT1 (GLAST), we were able to reduce glutamate in the cell culture media while expressing the NMDA receptor. Using binding studies, we were able to demonstrate a functional NMDA ion channel in these cells.

P29

Optiferrin Animal-Free Recombinant Human Transferrin Supports the Growth of Cells in Serum-Free Media

Steve Pettit[1], Mary Ann Santos[1], Tanya Tanner[1], Joseph Camire[2], Paula Decaria[2]
1: InVitria. 2: Thermo Scientific Cell Culture & BioProcessing

Cells require efficient iron delivery in order to support growth in serum-free formulations. Transferrin is a serum protein that transports iron into cells without the toxic effects found with elemental and chelated iron. Optiferrin, animal-free recombinant human transferrin, is a newly introduced product that provides effective iron transport into cells. We compared Optiferrin transferrin to serum-derived transferrin and another recombinant transferrin for its ability to support cell growth in serum-free media.

Optiferrin supported growth of Sp2/0 hybridoma equal to the plasma-derived and another recombinant transferrin and was more potent than bovine transferrin. The growth of cells was similar over the same dose response. Optiferrin also supported the growth of HL-60 cells, a human leukemic cell line dependent on transferrin, and performed equal to serum-derived transferrin in a dose response study. Also, Optiferrin supported the growth of HEK 293 and CHO cells in a dose response study.

These data indicate that Optiferrin recombinant transferrin provides efficient iron delivery to support cell growth in serum-free media.

Steve C. Pettit, Ph.D, is Director of Cell Culture for InVitria and leader of InVitria's cell culture product development group. He has authored numerous publications and has 20 years of cell culture experience.

P30

Optimizing bioprocessing of baculovirus for gene therapy: use of metabolomic tools for production and membrane processes for purification

MJT Carrondo, N Carinhas, V Bernal, C Peixoto, T Vicente, R Oliveira, P Alves
IBET – Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal

The first, seminal meeting of the PEACE Conference series held in 1992, targeted baculovirus as a tool for recombinant protein production, an area that has grown exponentially since then.

A decade and a half later, given their safety and large cargo load, the baculovirus themselves are being used as gene therapy vectors. In order to optimize and reduce the cost of production, two major areas of their bioprocessing have to be dramatically improved:

i) At the bioreaction level, the same cell density effect that reduces cellular productivities as the cell concentration at infection (CCI) increases in many infectious processes is apparent: extra viral production is almost stopped at CCI's above 2x106 cell/ ml. A thorough study of the central metabolism, coupled with metabolic flux analysis has allowed us to quantify, model and environmentally manipulate the metabolism of Spodoptera fugiperda cells. In particular, we could identify the branch points allowing for increased energetic metabolism and manipulate them to improve baculovirus production by one order of magnitude [1-3].

ii) The downstream processing of baculovirus is a nightmare, given its fragile, enveloped nature and its odd shape, with a length versus base ratio of approximately 8:1. A complete downstream process comprising three steps—depth filtration, ultra/diafiltration and membrane sorption—was designed presenting global recovery yields reaching 40% (at purities over 98%) and relying on technologies easy to transfer for processing scales under cGMP [4].

The key aspects of the optimized bioprocess will be presented and its relevance discussed.

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P31

Process Characterization and Validation of a Monoclonal Antibody Cell Culture Process Using a Quality by Design Approach

Brian Horvath, Melissa Mun, Michael W. Laird
Late Stage Cell Culture, Process Research & Development, Genentech, Inc.

The goal of Quality by Design (QbD) is to build processes which effectively produce products with consistent critical quality attributes (CQAs) by achieving better process understanding through the use of tools such as risk assessments and design of experiments (DoE). Initial process characterization study factors are chosen through risk ranking assessments based on historical development data, manufacturing history and scientific understanding. Analysis of statistically designed, multifactor, process characterization studies leads to an understanding of the critical process parameters (CPPs) that impact product CQAs as well as affect process key performance indicators (KPIs). CPPs are further characterized and validated to derive their design and control operating spaces for cGMP manufacturing. In cell culture process development, complex and large variations may be uncovered in multifactor study responses. This can be especially true when consecutive unit operations or responses are studied together in which the output of one operation is the input to the next. The work outlined here shows how Genentech implemented a QbD approach to characterize and validate a monoclonal antibody (MAb) cell culture production process. The approach, results and lessons learned will be discussed.

P32

Profiling Highly-Conserved MicroRNA Expression in Chinese Hamster Ovary Cells Using Microarray and Quantitative RT-PCR

Nan Lin, Trissa Borgschulte, Katherine Achtien, Angela Davis, and Kevin Kayser
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MicroRNAs have been revealed as an important aspect of global gene expression regulation and proposed biomarkers and cell engineering targets. MicroRNA expression in Chinese Hamster Ovary Cells remains understudied, despite the wide usage of these cells for therapeutic recombinant protein expression. In the present study, we used miRCURY Locked Nucleic AcidTM microRNA array as a screening tool for microRNA expression in CHO cells. The selection criteria of miRNAs for further profiling studies included positive hybridization signals, and validated predicted targets according to MIRBASE and TARBASE. Based upon these microRNA array results, we selected 16 miRNAs for further expression profiling. We performed quantitative RT-PCR of these miRNAs using Taqman® MicroRNA Assays. The cell lines used in the qRT analyses included parental DG44 CHO cells as well as four recombinant DG44-derived CHO lines producing a humanized IgG. Among the differentially expressed miRNAs, miR-23a, miR-221 and miR-222 were significantly down-regulated, whereas let7a was significantly up-regulated in at least two rIgG producing lines when compared to parental DG44 cells.

In addition, we investigated the expression patterns of miR-24 in various cell lines with differing dihydrofolate reductase (dhfr) genotypes and expression levels, in order to elucidate the relationship between DHFR expression and its known miRNA regulator in CHO cells. The following cell lines were included in the present study: parental DG44 [dhfr (-/-)]; parental CHO K1 [dhfr (+/+)]; parental CHO Duxb11 and five CHO K1 derived lines with one dhfr allele knocked out by Zinc Finger Nuclease (ZFN) technology [dhfr (+/-)]. We observed differential expression of miR-24 in these cell lines, and there was a loose correlation between miR-24 and endogenous DHFR relative mRNA levels. In the rIgG producing MTX-selected DG44 cell lines, with various copies of exogenous DHFR, miR-24 was also differentially expressed.

The results reported are the first steps towards profiling highly conserved miRNAs and studying the cloning difference in miRNA expression in CHO cells, and may shed light on using miRNAs in cell engineering.

P33

Protein and Membrane Supply for High Throughput Screening Assays by Transient Transfection and Baculovirus Insect Cell Expression

Georg Schmid et al.
F. Hoffmann-La Roche Ltd., Switzerland

The consistent and timely supply of purified proteins and cell membranes for assay development, high throughput screening and counter screening and/or selectivity assays is a mandatory step in the search for structurally new small molecules serving as leads for clinical candidate selection.

For several exemplary cases we will discuss data from recent expression campaigns for (1) a GPCR and a ligand-gated ion channel by large-scale transient transfection and (2) for a transmembrane protease and a membrane-associated enzyme activity from the baculovirus Sf9 cell expression system.

The following issues will be addressed:

- Optimization of transient expression of GPCRs and ion channels (e.g. host cells/ transfection reagent/ culture medium, bioreactor system, butyrate induction, etc.), monitoring of transfection efficiency by FACS

- Quality of membrane preparations obtained from traditional cell disruption methods (e.g. douncing, Ultraturrax) versus the use of N2 decompression for cell breakage

- Reproducibility of large-scale transient transfections alone and including the subsequent membrane preparation procedure

- Optimization of baculovirus post-infection times for efficient isolation of a membrane-associated enzyme activity by N2 decompression, reproducibility of membrane quality as assessed by binding assays

- Reproducibility of Sf9 cell fermentations and of the overall process including the multistep enzyme purification scheme in a research environment

P34

Recombinant Histone H3.1 Provides a Non-toxic, Surface-independent and Fully-defined Reagent to Enhance Retroviral Transduction

Pascal R. Beauchesne[1,2], Michelle Miller[3], Katherine J. Bruce[1,2], Sanja Sekulovic[3], Bruce D. Bowen[2], R. Keith Humphries[3,4], James M. Piret[1,2]

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The effectiveness of recombinant retrovirus-mediated gene transfer can be severely limited due to mass transport limitations, thereby requiring vector concentration steps and repeated exposures of the target cells to achieve desirable scientific and therapeutic outcomes. The low diffusivity (~6 x 10-8 cm2/s) and short half-life (~6 h) of retroviruses combined with various physico-chemical forces limit the encounter frequency of vectors and target cells. Cationic reagents such as protamine sulfate, an arginine-rich peptide, have been shown to improve retrovirus-mediated gene transfer by 4- to 8-fold in TF-1 cells. Histones, a group of cationic nuclear proteins rich in lysine and arginine residues, provide comparable enhancement. However, their use with retroviral vectors has been limited to mixed tissue-derived preparations, thus confounding the properties of the individual histone types. Initially, the effect of histones on the transduction efficiency of TF-1 cells was screened based on their arginine and lysine contents. A lysine-rich fraction (f1) predominantly composed of histone H1 and an arginine-rich fraction (f3) mainly composed of histone H3 extracted from calf thymus were added to TF-1 cells and retroviral vectors with a GFP reporter gene produced from PG-13 packaging cells. Unlike protamine sulfate that requires a tissue culture-treated surface, both histone fractions enhanced retroviral transduction independently of surface type. While the f1 histone fraction yielded a similar increase as protamine sulfate, the f3 fraction enhanced transduction by over 16-fold, a 3.5-fold improvement over protamine sulfate. These results were confirmed using unmodified human recombinant histones where H1o, H2A, H2B and H4 matched the effect of protamine sulfate, while the H3.1 variant provided a 10-fold increase in transduction. This recombinant H3.1 histone protocol was successfully applied to the retroviral transduction of mouse bone marrow enriched in hematopoietic stem cells (HSCs) by 5-FU pre-treatment, and was compared to a co-culture method. The addition of histone H3.1 was as effective as the co-culture method in transducing the HSC population where 50% of the donor-derived cells were GFP+ compared to 6% with no additives as verified by a long term reconstitution assay. This novel protocol based on the recombinant human histone H3.1 provides a fully-defined alternative with higher transduction efficiency and is compatible with sensitive primary cell populations such as HSCs.

P35

Single-batch manufacturing of Recombinant Human Polyclonal Antibody drugs

Anne B. Tolstrup
Symphogen, Elektrovej 375, 2800 Kgs Lyngby, Denmark

Polyclonal antibodies have proved to be very useful drugs for treatment of infectious diseases. Further, polyclonal antibodies have properties that make them well-suited for cancer therapy, especially when designed to harbor relevant and diverse target specificities. Whereas monoclonal antibodies have shown positive effects in several cancer indications, the field is moving towards combination therapy with the aim to enhance treatment efficacy. With the desire to develop target-specific recombinant polyclonal antibodies (rpAb) for human therapy, Symphogen has invented methods both for isolation of fully human target-specific recombinant antibodies as well as for consistent manufacturing of such drug products.

The SympressTM manufacturing technology enables highly consistent manufacturing of rpAb using a simple single-batch setup. In the first version of SympressTM a site-specific integration technology was used to generate stable polyclonal producer cell banks exhibiting sufficiently similar growth and productivity properties to allow consistent single-batch manufacturing. Symphogen's lead product, an anti-Rhesus D rpAb), which is produced using the first version of SympressTM, is currently in clinical phase 2.

However, the site-specific integration technology, which is based on incorporation of a single copy of the gene of interest, imposes limits to the production level of the stable cell lines. To improve titers and develop a more cost-efficient single-batch manufacturing mode, we tested a number of different approaches. Surprisingly, the most successful results were obtained using random integration in a new producer cell termed ECHO, a CHO DG44 cell derivative engineered for improved productivity at Symphogen. The compositional stability and the batch-to-batch reproducibility of rpAb produced by the ECHO cells has proven to be equal to or better than what had previously been observed using site-specific integration technology. Importantly, a very significant titer increase was obtained. The presentation will include a case story from a preclinical infectious disease project targeting the Respiratory Syncytial Virus (RSV) where expression of the anti-RSV rpAb is based on the new high-yield version of SympressTM.

P36

Stability analysis of humanized Rebmab 100 monoclonal antibody

M.L. dos Santos[1,2], A.A. Pimenta Jr.[1,2], A.C. Pavanelli[1,2], M.C.B. Schmidt[1], A. Garbuio[1], M.T.A Rodrigues[1], L.J. Old[3], O. Smaletz[2] and A.M. Moro[1]
1: Monoclonal Antibodies Laboratory, Instituto Butantan, SP, Brazil.
2: Recepta- biopharma, SP, Brazil. 3: Ludwig Institute for Cancer Research, NY, US

The LeY antigen is a blood group-related antigen highly expressed in over 70% of epithelial cancer (including breast, colon, ovary, and lung cancers) and is an attractive target for monoclonal antibody directed therapy. A Phase I clinical trial with humanized Rebmab 100 (Recepta-biopharma designation for licensed hu3S193 mAb) was performed in Australia by LICR with patients presenting advanced LeY positive cancer. Presently, a Phase II clinical trial with Rebmab 100 is under study in Brazil by RECEPTA-biopharma. To determine the effect of temperature shift on Rebmab 100, one vial of Rebmab 100 was aliquoted and submitted to freeze and thaw once, three or five times, and then analyzed by SDS-PAGE, SEC-HPLC, flow cytometry and BIAcore binding. We analyzed also hu3S193 vials stored at three different temperatures, -20°C, 5°C and 25°C, for 1 to 6 months. The absence of antibody aggregation was verified by SEC-HPLC; possible alterations in the Rebmab 100 binding affinity by flow cytometry and BIAcore analysis, and changes in the immune effector functions were evaluated by ADCC (antibody-dependent cellular cytotoxicity) and CDC (complement-dependent cytotoxicity) assays. A Rebmab 100 vial stored at -80°C was used as reference. The analysis of freezing cycles of Rebmab 100 samples did not show any difference from the reference sample. The analysis of Rebmab 100 samples at different temperature storage showed that only at the higher temperature tested (25°C) after 6 months of storage there was evidence of a slight reduction in the ADCC activity. Changes were not found in the other potency assays as CDC or the ability of the mAb to bind either to live cells (flow cytometry) or synthetic antigen (BIAcore analysis).

Financial support: FINEP, CNPq, RECEPTA-biopharma

P37

Stability of recombinant protein production in amplified CHO cells

Zeynep Duman[1], Alexandra S. Croxford[1], Steven G. Williams[2], Alan J. Dickson[1]
1: Faculty of Life Sciences, The University of Manchester, Oxford Road Michael Smith Building, Manchester, UK
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Therapeutic proteins require proper folding and post-translational modifications (PTMs) to be effective and biologically active. Chinese hamster ovary (CHO) cells are the most frequently used host for commercial production of therapeutic proteins due to their ability to perform the correct complex post-translational modifications. Due to the low productivity of these cell lines, DHFR-mediated gene amplification technique is extensively applied in order to increase protein production. However, some CHO cell lines are known to show a decrease in protein production during the time required for scale-up and this causes unpredictable loss of yield, time and money, and may jeopardize the regulatory approval of product. Ubiquitous Chromatin Opening Elements (UCOEs) are DNA elements naturally found upstream of specific housekeeping genes, which maintain an open chromatin structure, therefore diminishing instability of production by preventing transgene silencing over long term culture. Earlier studies have shown that UCOE-containing vectors give stable and high-level transgene expression. In this study we have examined the interaction between UCOE and DHFR-linked amplification in relation to cell expression stability. GFP engineered CHO-DG44 cell lines, amplified to 250 nM MTX with or without the inclusion of a Ubiquitous Chromatin Opening Element (UCOE) in expression constructs, were grown continuously for 80 days in the presence and absence of MTX selection. Growth characteristics, GFP and DHFR protein expression, over long-term culture were analysed using trypan blue exclusion and Western blotting techniques, respectively. UCOE cell lines were found to achieve higher cell numbers compared to non-UCOE cell lines. GFP and DHFR protein expression were observed to decrease over prolonged culture in both cell lines, in the absence of MTX selection. Future work will concentrate on molecular analysis of change in expression over long term culture. An understanding in this area will provide an improvement for cell line development and methods of recombinant protein production.

P38

Utilization of Site-Specific Recombination for Generating Therapeutic Protein Producing Cell Lines

Margie Campbell, Susanne Corisdeo, Clair McGee, Denny Kraichely
Centocor R&D Inc., Radnor, Pa

We have used the AttSite® Recombinase Technology, Intrexon, Blacksburg, VA, for the development of mammalian production cell lines expressing biotherapeutics of choice. This technology utilized specific DNA sequences (AttB sites) and proprietary recombinase enzymes to catalyze an insertion of a marker gene at locations in the host cell genome that are highly active for gene expression ('hot spots'). Subsequently, the marker gene was exchanged for CNTOX, a gene expressing a therapeutic protein of interest.

In first step of a two step process, a Chinese Hamster Ovary (CHO) host cell line was transfected with a targeting plasmid expressing a marker gene and ZsGreen (green fluorescent protein) between two AttB sites. Transfectomas were screened for the highest expression of the marker gene and further screened for chromosomal integration, selecting clones with one (or very few) copies per CHO genome. PCR was used to confirm that the selected transfectomas had intact AttB sites spanning ZsGreen and the marker gene.

In the second step of the process, an exchange plasmid containing DsRed (red fluorescent protein) and CNTOX, cloned between two AttP sites was introduced into the above generated transfectoma. Recombination between the targeting and the exchange plasmid was facilitated by a specific recombinase introduced at the same time and the process was monitored by the replacement of green fluorescence with red fluorescence. Finally, the exchanged pool of cells was plated and individual clones were screened for high expression of CNTOX. Results showed that CNTOX preferentially integrated into the transcriptionally active site or 'hot spot', previously occupied by the marker gene, resulting in clones with uniform high-levels of protein expression.

Using the AttSite® Recombinase Technology, it is possible to generate candidate production cell lines with significantly less screening, which may reduce the time required for the development of production cell lines.

WORKSHOP SCHEDULE
Chair: Girish Shah, GlaxoSmithKline

Monday September 21st: 13:30 - 16:00

13:30 - 14:00 **Workshop 1: Invitrogen -** Balwant Patel, Director Market Development, Invitrogen
Latest Protein Expression and Protein Gel-Electrophoresis products from Invitrogen

14:00 - 14:30 **Workshop 2: Crucell -** Gregory Zarbis-Papastoitsis, Percivia, LLC, USA
Advances in manufacturing of biologics using PER.C6® a human cell line

15:00 - 15:30 **Workshop 3: Invitria -** Steve Pettit, Ph.D, Invitria, USA
Zap-CHO Media Supplement Improves the Growth Kinetics and Productivity of CHO cells

15:30 - 16:00 **Workshop 4: Ajinomoto -** Hiroshi Izui, Ajinomoto, USA
The CorynexTM Protein Expression System: a novel approach for protein production

Invitrogen - Latest Protein Expression and Protein Gel-Electrophoresis products from Invitrogen

Balwant Patel, Director Market Development

The presentation will provide an overview of the following new products and technologies: Rapid expression of soluble (active) membrane proteins using the MembraneMAXTM Expression Kits; new Bac-to-Bac® TOPO® Kits, for 5 min, high throughput cloning and expression of PCR generated DNA fragments in insect cells; PichiaPinkTM, a new expression system for high yield protein production using pichia pastoris; the NeonTM Transfection Device, a cutting edge solution for transfection of primary, and other difficult to transfect cells; and the BenchProTM4100 Device for fully automated walk-away Western processing.

Crucell - Advances in manufacturing of biologics using PER.C6® a human cell line

Gregory Zarbis-Papastoitsis
Percivia, LLC, USA

Recent breakthroughs using the PER.C6® cell line have resulted in high volumetric productivities, greater than 10 g/L in Fed Batch and 27 g/L in XD®, while achieving high cell densities. These productivities resulted from a combination of the intrinsic properties of the cell line (resistance to apoptosis, promotion of growth and productivity), and advances in cell culture development. The higher titers achieved with PER.C6® are also accompanied by post translational modifications that mimic human plasma derived proteins.

Extreme productivities have generated pressure on downstream processing requiring purification methods with higher capacities and reduced cost. In this presentation we will discuss some of the capabilities of the cell line and opportunities for manufacturing based on single use technologies and high capacity purification steps. The economic benefits of these processes will be discussed.

Invitria - Zap-CHO Media Supplement Improves the Growth Kinetics and Productivity of CHO cells

Steve Pettit, Ph.D
Invitria, USA

Improving the growth kinetics and productivity of CHO (Chinese hamster ovary) cells is a major challenge in the bioprocessing industry. We evaluated Zap-CHOTM animal-free media supplement for its benefit in CHO culture. Zap-CHO is a newly introduced product that consists of a cocktail of defined protein factors designed to improve CHO culture in modern media formulations.

Zap-CHO was tested in six (6) commercially available CHO media formulations on a CHO K1 line. Zap-CHO improved the growth kinetics in every formulation tested. Cell growth, measured by Integral of Viable Cells ("IVC"), improved an average of 190% across the six (6) media, ranging from 98% to 372%. Maximum cell density typically increased 50%. In addition, Zap-CHO improved cell viability at the end of the batch, typically extending the cell viability during the batch by 2-4 days. Across the six (6) commercially available media formulations, productivity increased 69%. Detailed kinetic analysis in a commercial formulation supplemented with 0.5 grams/liter of Zap-CHO showed an increase in productivity of 63% and an extension of cell viability of four (4) days.

These data indicate that Zap-CHO is an effective tool to improve the growth kinetics and productivity of CHO cells in modern media formulations.

Ajinomoto - The CorynexTM Protein Expression System: a novel approach for protein production

Hiroshi Izui
Ajinomoto, USA

Ajinomoto has a century long heritage of R&D investment and innovation with 50 years of pharmaceutical experience. Today, Ajinomoto is recognized as the unrivaled leader and premier supplier of pharmaceutical grade amino acids. Ajinomoto's CorynexTM Recombinant Protein Expression System is a streamlined and more efficient process to express proteins. The patented system overcomes traditional microbial expression difficulties by secreting correctly folded, active proteins directly into the growth media. This system reduces costly and time consuming downstream purification steps which decrease production costs and accelerate speed to market for Ajinomoto's pharmaceutical and biotech partners. A "toolbox" of technologies lends flexibility and helps to broaden the potential of CorynexTM to express a wide range of proteins. The system has been used to express a variety of proteins from industrial use enzymes to cell culture growth factors and proteins for pharmaceutical applications.

SPECIAL PRESENTATION

Wednesday September 23st : 16:30

Proposed Laws Regulating the Approval of Innovative and Follow-On Biologics

Verne A. Luckow, Ph.D., J.D.

FDA and PTO regulations are complex, requiring careful preparation of documents that are reviewed by regulatory officials and patent examiners according to procedures that often take years to complete. Despite attempts by Congress to fairly compensate inventors or assignees for delays which occur during periods of review by the FDA or the PTO, products regulated by both agencies may be subject to overlapping periods of data (market) exclusivity, and patent exclusivity, the normal term of which may be adjusted, extended, or disclaimed. Predictable, fixed terms of exclusivity, have given way to variable overlapping terms, determined by the starting and ending dates of dozens of review periods, which may a single time, more than once, or rarely before a regulatory agency. Variable periods, reviewer discretion, and complex multi-agency rules, all invite challenges, by innovators seeking to prolong the right to exclude others from selling their products, and by generic manufacturers, seeking to sell competing products, that fuel healthy competition in the market. Striking the right balance, to avoid industry- or size-specific regulations, is not an easy task. Recent challenges to the interpretation of PTO rules that affect the term of a patent, if upheld, plus proposed new rules affecting the regulation of biopharmaceutical and small molecule products, if implemented, will dramatically alter the procedures needed to discover, develop, approve, and market these products. Many business strategies, reflected in research and development plans, license agreements, and litigation, depend on a clear understanding of all the issues affecting the regulatory approval process and the term of a patent, to facilitate decision-making by executives seeking to protect their products in competitive business environments around the world. There is currently a lot of debate in Congress over the provisions in proposed bills regulating the length of various data exclusivity provisions that are designed to protect an innovative biopharmaceuticals “reference compounds” from competition by manufacturers offering “follow-on biologics”. While a 12 year period of data exclusivity is currently favored, many of the proposed laws leave much to be desired with respect to the definition of key terms, such as identity, functional similarity, and immunogenicity, and the extent to which the FDA has control regulating the approval of biopharmaceuticals on a case-by-case basis. Areas likely to be litigated, between manufacturers and the FDA, and between innovator and follow-on manufacturers, will be briefly discussed, along with strategies for minimizing delays, and maximizing data exclusivity and patent term protections, that add value to companies seeking to bring products to market that meet unmet medical and economic needs in today’s challenging global economy.

AUTHORS INDEX

List of Participants

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dos Santos, Mariana	Instituto Butantan/Recepta Biopharma
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Nielsen, Lars	Symphogen A/S
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Zarbis-Papastoitsis, Gregory	PERCIVIA, LLC
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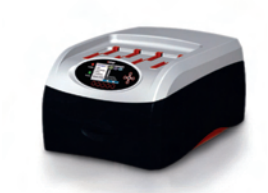

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