Word from the Chairman

Dear members and friends of ESACT,

ESACT in Transition

At the last ESACT general assembly in Dublin, we have suggested that ESACT follows a transition from a “Meeting Society” running one of the world’s most successful conferences in animal cell technology to a “reference society for learning cell culture technology”. In that sense ESACT has committed to support a variety of learning and teaching events. Our new series of animal cell technology courses, of which two have been initiated during the past year, will hopefully be the core success: i) Quico Gòdia and Paula Alves have designed a “Animal Cell Technology Course” that will take place next October 2011 and ii) Hansjörg Hauser, Quico Gòdia and Tobias Cantz have organized a course on “Regenerative Medicine – From Bench to Bedside” in 2010 that will be repeated in 2011.

ESACT has also been supporting iii) Sabine Geisse and Holger Heine et al. in their continued organized European BioTechnology Workshop series and the iv) Latin-American Community working with Animal Cells in their Animal Cell Biotechnology Courses related with Biopharmaceuticals Production, both of which have also become a reference think-tank for animal cell technology. This is to say thank you to those organizers and all lecturers of these various courses for the extra efforts. At the same time we hope that the example of these colleagues will kick-off enthusiasm of others to help organize more courses or “donate” their time to serve as lecturers – please remember ESACT is a “volunteer society” dependent on activities of its members.

Besides these new learning and teaching activities, ESACT has faced two other major transitions in the past years. Christophe Losberger, who was running the ESACT office will refocus his professional career and will, much to our regret, step down. For the past 13 years, Christophe has been the brain behind about everything which gave ESACT a professional administrative face: he designed the webpage, initiated the Job Information Network (JIN) together with Stefanos Grammatikos, transferred our membership (fee) database into an electronic format, reshaped ESACT voting processes to be all online and, he organized the content, layout and dispatch of our newsletter. Christophe had a dramatic impact on how the society developed in recent years. ESACT cannot thank him enough for his achievements. At the same time we are looking forward to the collaboration with our new head of the ESACT secretariat, Dr. António Roldão, and wish him all be best in this central ESACT position.

…And one more transition – After 35 years ESACT has become a “legal society”. Under the leadership of Hansjoerg Hauser we have worked on this transition during a wonderful XC meeting in Bruxelles, last year. More coverage on this event in the following paragraph, by Mike Comer.

ESACT Constitution and Legal Entity Status

As all Members and most of its’ Conference Delegates are aware “ESACT” was established in 1976 by a handful of experts in the field who felt representation, discussion and communication of their skills were necessary to progress their scientific expertise more effectively. The “Society” has greatly evolved since then from this “handful” to more than a thousand of you, the experts in the field. Since then, although in terms of
scientific endeavor a relatively short time, the field has positively developed beyond all expectations and forms the basis of incredible advances in human knowledge and modern health care. The political, legislative and economic environment has also developed in an equally dynamic fashion although perhaps not so positive in many of its aspects. The world is a very different place than in 1976 and restrictions and legislation have become exceedingly complex in the control of finance and operations on a national and international level. These developments have been a concern and a serious consideration and debate for the Executive Committee for some years now. Therefore, the decision was taken to re-establish ESACT in a more appropriate form to address its needs and infrastructure in indisputable compliance with current and potential future legislation, socio-economic and political developments.

In short, with the help of many experts, the Executive Committee is proud to announce that ESACT e.V., your society, is now established in Germany as an unequivocal Legal Entity. The ESACT Constitution, that as governed ESACT from its beginning, had to be adapted in order to meet the legal demands required to establish a Society in Germany. A “new” version of the Constitution has been drafted by lawyers from a German legal firm who are experts in this type of brief (Lothar Boelsen Wirtschaftsprüfer, Prof Dr. K Schwantag, Dr Kraushaar GmbH).

The task has been a daunting one since there were very many scenarios, options, pros and cons to consider and the decision for Germany as the “country headquarters” was taken after much and intensive deliberations indeed, also in consultation with one of the original founding members and many of those who have supported ESACT from its beginning. Germany provides a comprehensive and a central position within Europe to deal best with the requirements of the Society now and in our far-reaching future. We would now like to introduce you to your old but “newly” established European Society for Animal Cell Technology e.V.. The Executive Committee look forward to your help and full support in ratifying our decisions during the ESACT 2011 General Assembly that will take place 17th May - 12:30, at the forthcoming 22nd ESACT Meeting in Vienna.

Mike Comer,
on the behalf of the Executive ESACT Committee

ESACT Courses

Animal Cell Technology Course (3-6/Oct/2011)

ESACT is organising the first Course on Animal Cell technology. This is an introductory course, providing an overview of the field, from the more basic aspects to the final applications. It should be of interest to those starting their research activity in Animal Cell Technology, both from Academia or Companies. It is also of interest for those wishing an up-date of the state-of-the-art of Animal Cell Technology in a short overview Course.

The Course is planned in an intensive four day schedule, with a number of participants limited to a maximum of 30, in order to facilitate the interaction among them and with the lecturers. Lecturers will stay for most of the Course duration. The Course comprises lectures and study case analysis.

The Course will be held in hotel Terramar, in a small town named Llafranch, in the heart of Costa Brava, Girona, Spain, providing a cozy and relaxing atmosphere for those attending. The hotel is right on a very nice sandy beach. The Course will take place from 3 to 6 October.

The following topics will be covered:

5. Downstream processing. Separation and purification technologies.
8. In-process analytical technology.

Course organizers are Paula Alves (IBET, Lisbon) and Francesc Gòdia (UAB, Barcelona). Confirmed lecturers include Terry Papoutsakis (University Delaware), Manuel Carrondo (IBET, Lisbon), Hansjörg Hauser (HZI, Braunschweig) and Ashraf Amanullah (Genentech, California).
Registrations to the Course will be done through the ESACT office (roldao@esact.org). More information will be available soon at ESACT web site (www.esact.org).

Stem Cell Technology Course (29Sept-20Oct/2011)

Stem Cells – from Generation to Application

Following our successful course in 2010, we are again organizing an ESACT Stem Cells Course in Llafranch, Spain. The field of stem cell understanding and technology is in an ongoing transition phase from academy to application. This introductory course will provide an overview of the field, from the basic aspects to the final application. It should be of interest to those starting their research activities, both from Academia or Companies. It is also of interest for those wishing an up-date of the state-of-the-art knowledge in a short course.

The organizers are Tobias Cantz (REBIRTH Junior Research Group “Stem Cell Biology”, Hanover), Francesc Gòdia (local organizer at University of Barcelona) and Hansjörg Hauser (Helmholtz Centre for Infection Research, Braunschweig).

Renowned lecturers will cover the fields of:

1. Generation of iPS cells
2. De-differentiation, differentiation, trans-differentiation
3. Isolation of murine and human stem and precursor cells
4. Properties and quality of cells
5. Cultivation: small and large scale
6. Bioreactors
7. Regulatory aspects, GMP
8. Patenting, business and career options
9. Application areas: Human treatment and pharmacology

For further information please refer to the ESACT website (www.esact.org) or www.helmholtz-hzi.de/stemcells. The website will be continuously updated.

The ESACT Animal Cell Technology Course will be organized immediately after the Stem Cells course, at the same location. Registration procedure for one or both courses will open soon.

Workshops at ESACT 2011 in Vienna

Nicole Borth, BOKU University, Austria

At the upcoming ESACT 2011 Meeting in Vienna an unusually high number of workshops will be offered on Sunday May 15th in the Vienna Hofburg Conference Centre. These workshops are free to all participants of the ESACT conference and will provide overviews over very diverse topics. Three of these workshops (A, B and C) are science driven, including workshops on Recent Advancements in Viral Vector Manufacturing, the exciting CHOgenome.org workshop and one on the use of FC Fusion Proteins as Therapeutics. In addition there are 8 workshops organized by different technology providers presenting their newest developments. The workshops will run in three parallel streams during Sunday, thus unfortunately you will have to pick and choose!

Workshop A: Recent advancements in Viral Vector Manufacturing

8:30-10:00 - Organised by Amine Kamen, Bioprocess Centre, National Research Council, Canada

Viral vectors are extensively used as delivery systems for gene and cell therapies, oncotherapies and vectors for display or expression of antigens in different vaccination strategies. Also, viral vectors are important tools for acceleration of drug discovery.

Over many years, developments in cell culture technologies have been critical to enable mass production of viral vectors and have greatly contributed in facilitating pre-clinical and clinical trials for therapeutic applications. However, progress reports are
confined to specialized conferences in these fields and results are published in journals often not accessible by Animal Cell Culture Technologists. The purpose of this workshop is to review the main technological advancements in the field of cell culture-based manufacturing of viral vectors including:

- Adenovirus and Adeno-Associated viruses
- Lentiviruses and retroviruses
- Baculoviruses and other enveloped vectors
- Other vectors for vaccination

This workshop will address novel approaches in upstream and downstream processing as well as critical developments in quantification of viral particles, and process intensification. Short presentations will be delivered by experts from academia and industry to inform the cell culture community about key advancements in the field of viral vector manufacturing. We also wish to engage the audience in discussing the remaining challenges that cell culture technology can address in further advancements in Gene and Cell Therapy and Novel Vaccine development.

Workshop B: CHO Genome Workshop

10:15-12:45 - Organised by Mike Betenbaugh, Nicole Borth and Kelvin Lee

During the past year, efforts to obtain useful sequence data information for CHO cells have increased significantly, thus stressing the necessity for easily accessible databases and sophisticated tools for analyses of biological data sets. The web site, www.CHOgenome.org was founded as a CHO community-based effort to establish a single site for combining and housing CHO related tools. This site will include publicly available genomic, cDNA and microRNA sequence information as well as other datasets in a format that facilitates their exploitation in biological and bioprocess research. The workshop will highlight the currently available data sets and their use, followed by a discussion on the expanding needs of the worldwide scientific community with respect to the CHO genome. Those interested in facilitating the use and future developments for this website are encouraged to attend and participate.

Workshop C: Fc Fusion Proteins: A Growing Class of Therapeutics

14:00-15:45 - Organised by Steven Chamow, Ph.D., S. & J. Chamow, Inc.

The potential therapeutic value of many proteins - including enzymes, cell-surface receptors, cytokines and peptides - can be realized by fusing these proteins to the Fc region of human immunoglobulin G. Of the 30 mAb products approved as human therapeutics in the USA to date, 4 are Fc fusion proteins, and many more are in clinical testing. Considerations in fusion protein design and production will be presented.

Workshop 1: Massively Parallel Sequencing (MP-Seq): a versatile tool -for Adventitious Agent Detection and Virus Discovery – and for Identification of High Producers and Validation of Genetic Stability

8:30-10:00 - Organised by David Onions, Bioreliance

Recent contaminations of manufacturing processes by porcine circovirus and vesiviruses have highlighted the need for broadly based and rapid methods to detect adventitious agents in cell banks, virus seeds and bulk product (drug substance). Massively parallel sequencing (MP-Seq) is a powerful new method for the identification of viruses and other adventitious agents, without prior knowledge of the nature of the agent. BioReliance have developed MP-Seq methods to detect free viruses in raw materials and fermenter samples. Our application of this technology has resulted in the discovery of a new parvovirus in bovine serum capable of infecting human cells and we have used this technology in the investigation of fermenter contaminations.

In some cells, the genomes of latent or transforming viruses may be present in a cell but no virus particles are produced. However, latency associated or transforming gene mRNAs are expressed. We have developed a method to identify these latent viruses by sequencing the total transcriptome of the cell and applying an algorithm to identify the viral specific transcripts. Enormous amounts of data (~400Mb) are generated in this process and a robust algorithmic process is required to analyse the data. Using this method we have been able to identify a new retrovirus expressed in Vero cells and we have identified nodavirus and errantivirus contamination of insect cells. The definitive nature of the methodology provides considerable reassurance that cell banks are free of unexpected contaminating agents.

We are also developing MP-Seq to provide rapid end points in in vitro virus detection assays. We have shown that we can detect virus infection as early as day 4 post-
infection with MP-Seq while conventional methods may require 14 or 28 days to reveal infection.

**Workshop 2: Using a Rational Approach to Develop Cell Culture Manufacturing Processes**

8:30-10:00 - Organised by Irvine Scientific

Successful upstream manufacturing processes for new biopharmaceutical products are dependent on two key parameters; the development of optimal cell culture media formulas (both growth media and feed solutions), and on the development of manufacturing protocols which result in consistent yields and product quality. Optimization of cell culture media formulas can be accomplished using a variety of approaches, each featuring certain strengths and weaknesses. Various methods and tools will be discussed and compared, including media library screening, component heat mapping, metabolic profiling, DoE optimization of components individually and as groups, and metabolomics, using data from actual case studies as examples. The most effective approach often involves combining several methods and tools chosen in combinations to fit particular project needs. The role of a media partner in supporting innovation during cell culture manufacturing process development will also be discussed.

**Workshop 3: BD Mosaic™ MSC SF: A New, High Performance Serum Free Culture Medium for the Expansion of Mesenchymal Stem Cells**

10:15-11:45 - Organised by Dr. James W. Brooks, BD Advanced Bioprocessing

Cell based therapeutics using Mesenchymal Stem Cells (MSC) are emerging for the treatment of a wide range of acute and degenerative human diseases. BD Mosaic™ MSC SF is a unique, high performance medium, which, through several key features, enhances MSC expansion with reduced culture time, labor, and medium requirements.

**Workshop 4: Strategies for Viral Risk Mitigation in Novel CHO Platform Expression Systems**

10:15-11:45 - Organised by Kevin Kayser and scientists from the Cell Sciences and Development group at SAFC®

ZFNs are a class of engineered DNA-binding proteins that facilitate targeted editing of the genome by creating double-strand breaks in DNA at user-specified locations. They can be harnessed to generate precise genomic edits resulting in enhanced cell lines, including the development of Chinese hamster ovary cell lines, with targeted gene deletions (knock-outs), integrations (knock-ins), or modifications. We will discuss the development of catalog ZFNs, unique customer ZFN applications and a series of Chinese Hamster Ovary (CHO) cell lines developed by SAFC (trademarked as CHOZn), for use in the Biopharmaceutical industry. We will also show how we have assembled a “Platform Process” to produce Biopharmaceutical industry levels of recombinant protein. This platform process includes a CHOZn parental cell line as well as a platform medium and feed that have been designed to work in harmony. The platform process is selected to deliver optimum performance in fed-batch bioreactors. Medium and feed formulations were developed using chemically defined and animal component free raw materials that have a proven track record for Sourcing and robust manufacturability.

**Workshop 5: Towards a fully single use protein production facility**

12:30-14:00 - Organised by Sartorius

During the last decade the advent of larger scale single use process solutions have changed our approach to making proteins from cell cultures dramatically. Driven by a growing pipeline of biopharmaceutical drugs in development and the cost pressure that the Pharma industry is experiencing, more and more companies are adopting single use bioreactors up to and beyond the 1000L scale. A similar trend can be observed in downstream processing although probably less matured yet. Membrane adsorption lends itself to single use applications with the potential of combining cell removal, clarification and capture into one unit operation. A number of companies have already taken the step and implemented hybrid or even single use production facilities for monoclonal antibodies or vaccine production. Scaleability of the process and consistency of results from bench-top bioreactors used in process development and as scale-down models in process validation to commercial scale production bioreactors is a key prerequisite for successful drug development. Therefore, ideally the design of the single use bioreactors should be as close as possible to the full scale systems to reduce the number of variables that may influence product quality and process performance. Finally, with all this different disposable solutions used in GMP production, not only the vendor - user
relationship has changed but also the approach to validation of single use equipment such as bioreactor bags and containers for process fluids.

**Workshop 6: From Molecule to Market: PD Solutions from Life Technologies**

12:00-14:00 - Organised by Life Technologies

Freedom™ CHO platforms: Gene to clone in 3-5 months, using 1 FTE, achieving 1-3 g/L, with no milestone or royalty payments.

Media and Feed platforms: Control of cell function by nutrient and process integration

Highly Sensitive PCR Based Assays: Rapid Detection of Cell Culture Contamination

**Workshop 7: Rethinking Media Supplementation: Identifying Bioactive Molecules that Drive Protein Production**

14:15-15:45 - Organised by Elizabeth C. Dodson, BD Advanced Bioprocessing

Serum. Hydrolysates. Chemically-defined. Rethinking media supplementation requires the identification of molecules that drive growth and protein production. Biochemical deconstruction of hydrolysates coupled with high resolution analysis was used to identify these molecules. Using DoE, a chemically defined media supplementation has been developed to substitute for yeast extract peptone.

**Workshop 8: How QbD is Changing Upstream Bioprocess Design**

14:15-15:45 - Organised by William G. Whitford, Thermo Scientific Cell Culture & BioProcessing

In light of QbD, PAT, and process platform imperatives, bioproduction process design now requires capabilities and capacity beyond essential facilities and personnel. The goals of producing high levels of quantity product in a robust and flexible production process require capabilities beyond even beyond subject matter experts with access to a repertoire of reference formulations. In-house technologies now demanded include a full complement of cell and culture media analytics and HTS capabilities. In most cases, some levels of product quality and attribute assays beyond simple product level quantitation are highly recommended. Process optimization toward highly regulated manufacturing can also draw upon such capabilities as regulatory certifications, quality management systems, and qualified raw materials sourcing. Modern demands for increased process understanding, CPP determination, and robust design space development virtually require assess to qualified scale-up and technology transfer equipment and methods rather early on in process development. Increased demands in process implementation efficiency are best supported by early consideration and testing of appropriate product containment and transfer technologies. The case-studies presented here illustrating such approaches are the fastest way to communicate the art and science of modern process design.

**Article Contribution**

The Exciting CHO Genome Era (CHOgenome.org)

Michael Betenbaugh, Johns Hopkins University and Kelvin Lee, University of Delaware

A new day is dawning on the biopharmaceutical community and the landscape of mammalian biotechnology will likely be forever changed in the year 2011. The Chinese hamster (**Cricetulus griseus**) ovary (CHO) cell line was first isolated more than 50 years ago and is currently used for production of biopharmaceutical proteins ($100 billion USD in annual revenue). CHO cell lines have been used in a variety of biomedical research contributing to developments in pharmacology, toxicology, cancer biology and treatments (Jayapal et al. 2007). More recently CHO cells have become the dominant production cell line for biopharmaceuticals in the world. Nearly 70% of all recombinant biotherapeutics are produced in CHO cells (Wurm 2004, Walsh 2010). Biopharmaceutical production will continue to provide the pharmaceutical industry with tremendous growth in the next several years, enabling continued research into new medical treatments and the expansion of manufacturing facilities (Jacob et al. 2009, Walsh 2010). CHO cells have become a dominant host system due to their ability to produce recombinant proteins with therapeutically acceptable glycosylation patterns and other post-translational modifications and due to the presence of powerful gene amplification systems. However, while affiliated technologies continue to progress at an unrelenting pace, advances in CHO cell innovation have stagnated in recent years. We believe that this is about to change.
Despite its scientific and economic importance, there is not yet a publicly available genome sequence for CHO cells. Previous efforts to develop CHO genomic resources include EST and transcriptome (Kantardjieff et al. 2009), microRNA (Johnson et al. 2011, Hackl et al. 2011), and low-coverage genome (Hammond et al. 2011) sequencing. Even with the published map of the CHO mitochondrial genome (Genbank DQ390542) and the annotation of dozens of CHO genes and microRNAs in public databases and the literature, the closest sequenced and annotated organisms are mouse and rat, both of which demonstrate significant differences from hamsters based on cDNA sequences (anecdotally investigators have observed about a 2-3% homology with these rodents). This limits the direct application of sequence-based molecular tools in bioengineering and cell line development efforts.

Fortunately, there are now parallel efforts ongoing at multiple international centres of excellence in Asia, U.S., and Europe to address this glaring deficiency and bring the CHO community into the genome era. To help accommodate the rapid output of information that is likely to emerge, to help facilitate communication between groups, and to provide a home site for ongoing multi-pronged efforts, an international CHO community genome project is now under development at the web site www.CHOgenome.org. The goal is to develop a one stop shop for the CHO community to learn about the latest genome efforts and publicly available data similar to what is available to the fly, mouse, yeast, and human genome communities. Model organism databases (MODs) are an essential tool for the collection, curation, and dissemination of genetic data for the research community. Well-established MODs have been developed for several model organisms including mouse (www.informatics.jax.org), rat (www.rgd.mcw.edu), Drosophila (www.flybase.org), and yeast (www.yeastgenome.org). As next-generation DNA sequencing technologies continue to improve, the time and cost of sequencing genomes is decreasing, resulting in the rapid development of genome-scale data sets for non-model organisms.

The recent rapid advances that are about to emerge on the CHO genome front were vividly on display during a recent symposium entitled, CHO Genome Workshop, held as part of the 5th International Conference on Genomics November 15-18, 2010 in Shenzhen, China. The workshop opened with remarks from Kelvin Lee who gave a history of how the community came together and Bernhard Palsson who provided context on the use of CHO cells in the biotechnology industry. Specific research talks were then given led by a keynote address from Xu Xun of BGI, who provided the first ever detailed description of the CHO-K1 genome and described many features of the CHO genome. Takeshi Omasa provided an overview of physical mapping and karyotyping efforts on CHO, highlighting the dynamic nature of the chromosomes. Steve Quake discussed efforts to characterize the CHO exome using a variety of techniques and assembly of these data in the context of mouse data. Nicole Borth presented an analysis of CHO microRNA including some possibly new microRNAs that have not been reported previously. Gyun-Min Lee gave a detailed overview of the use of proteomic methods to characterize CHO cells and particular CHO phenotypes of interest. Lars Nielsen provided a telling description of how genome-scale flux analysis models can be developed - something that is now possible with a CHO genome. Finally, Bernhard Palsson who is working together with BGI on one of the CHO genome sequencing projects, elaborated on specific elements of the CHO-K1 genome including a comparison to mouse, rat, human genome and identification of critical functional genes. Finally, Mike Betenbaugh and Kelvin Lee led a discussion of next steps going forward for the community. These include the importance of sequencing the hamster and other cell lines as well as having a simple framework for the community to interact with these data. In addition to these groups, a number of other groups are hard at work on various CHO genome projects including the long term efforts of Wei-Shou Hu in collaboration with Miranda Yap as well as a new initiatives emerging in the UK.

Where do we go from here? Once the genome sequence becomes available, a thousand follow-up questions emerge about how to organize the data and what additional experimental and computational tools can be brought to bear on the CHO genome. We envision www.CHOgenome.org playing a key facilitating role to...
inform and educate the scientific community on these advances. For this community effort to generate significant benefit, collaborations between these research groups and groups with bioinformatic expertise will be beneficial. To maintain community engagement and responsibility, relevant data will be stored in a publicly accessible database which will both provide access to the information, but also provide the community with the opportunity to participate in ongoing updates and to develop ancillary genome-scale tools. We envision the development of an organism database for CHO in the coming years to house and organize these tools. As the next step forward, the CHO genome community will come together for a special workshop at the upcoming European Society for Animal Cell Technology conference in Vienna Austria, May 15-18, 2011.

References:


Jacob NM et al. (2009) “Using genomic tools to improve the production of biologics.” Chemical Engineering Progress 105, 35.


ESACT Office - News

Since the 1st of April ESACT has a new Office. Due to a change of orientation in his career, Christophe Losberger (Merck Serono, Switzerland), has step down from the ESACT office.

The ESACT Executive Committee would like to thank Christophe for his commitment to the Society and all the help and support provided during the last 13 years.

Christophe Losberger put his first finger in the ESACT in 1998, when Alain Bernard, his line manager at that time, asked him to setup the website for the ESACT 1999 meeting at Lugano. During the Lugano meeting he met Bryan Griffiths (one of the ESACT founders) that asked him to take care of the ESACT website. Christophe participated actively in the organizing committees of the 3 following ESACT meetings (Tylosand 2001, Granada 2003 and Harrogate 2005). In 2003, he helped Stefanos Grammatikos to push JIN (the Animal Cell Technology career center); together they developed a dynamic web based software currently available at the ESACT website. Other duties came with time namely the administration of the ESACT member’s database (that become an online system in 2004) and the edition of the ESACT newsletter (from 2007 to 2011).

António Roldão, a former PhD student of Manuel Carrondo’s lab, IBET, Portugal and currently doing a Pos-doc at Jens Nielsen’s lab, Chalmers University of Technology, Sweden, will replace Christophe Losberger.

Paula Alves
ESACT Secretary

ESACT Newsletter May 2011

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