

Newsletter

January 2005

Editorial

Dear Readers,

As we slip into the holiday season, once again we give thanks for the many achievements that have transpired in the last year. In this issue, Otto gives us his Chairman's message of the 19th ESACT meeting, election of new committee members and advertising on JIN; and Alain proposes a constitutional change.

Merlin and I bring to you news about a recent UK Singapore science meeting, stem cell research at BTI and an update of stem cell research in the UK from Glyn Stacey. There follows a review paper on production of proteins by Florian Wurm and a letter from John Aunins from Merck. There is also a report of the 3rd Rec-Protein meeting written by Alain Bernard *et*

al. Then we felt that we should lighten and brighten the end of the old year with a healthy dose of jokes!

May this Christmas be a joyous and meaningful time for you and your loved ones. Blessings!



Chief Editor, Steve Oh

P.S. While completing this letter, a 9.0 Richter earthquake struck much of Asia. For those willing and able to help, I recommend these rapid response aid agencies:-

<http://www.worldvision.org/>
<http://www.redcross.org/>

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A Word from the Chairman

Dear ESACT Member,

The end of another year approaches and everybody is hectic preparing themselves for Christmas and the New Year. Thus, I wish you all the best and relaxing Christmas holidays.

However, time does not stop, and the next ESACT Meeting is approaching rapidly. The organization of the 19th ESACT-Meeting, which will be held in the nice city of Harrogate in North Yorkshire between the 5th and 8th June 2005, is well on the way (thanks to Rod Smith from CTM BioTech in Cambridge). The submission deadline for the abstracts is over now, and the Scientific and ESACT Executive Committees will decide on the final scientific programme during a joint session in February 2005.

Six scientific sessions are planned for 19th ESACT-Meeting:

1. Transcription to Secretion (Chair: Martin Fussenegger), keywords: cellular mechanisms, siRNA, toxicology screening, expression systems, post-translational modifications.

2. Therapeutic Cell Engineering (Chair: David Venables), keywords: transgenic cells, whole cell therapy, whole cell vaccines, implantation methods, novel methods.

3. Gene Medicine (Chair: Martin Fussenegger), keywords: gene delivery, viral vectors, non-viral vectors, gene regulation, epigenetic imprinting

4. Cells to Tissues (Chair: Glyn Stacey), keywords: embryonic stem cells, adult stem cells, tissue engineering, cell differentiation, artificial organs, tumourgenicity

5. Protein Products (Chair: Mohammed Al-Rubeai), keywords: therapeutic proteins, fusion proteins,

monoclonal antibodies and fragments, vaccines, recombinant proteins for diagnostics

6. Process Technology (Chair: Jürgen Lehmann), keywords: scale-up, process monitoring and design, downstream integration and application, productivity, high throughput, stem cell propagation, regulatory aspects.

The following Keynote Speakers are confirmed:

- A. Aguzzi (Inst. Neuropathology, University Hospital of Zürich): Molecular biology of the prion
- A. Bradley (The Wellcome Trust Sanger Institute): Genetic screens in embryonic stem cells
- B. Buckland (Merck & Co. Inc.): Recent advances in cell culture used for manufacturing vaccines
- J. Birch (Lonza Biologics plc): Challenges and opportunities in the large scale production of therapeutic proteins
- W. Berthold (IDEC Pharma): title to be confirmed

More information can be found on the web site of the 19th ESACT Meeting (www.esact2005.org). As usual during each meeting a general assembly will be organized. The general assembly will take place at the conference centre in Harrogate, of course, during lunch time on the 7th of June 2005. The agenda is not yet finalized, however, the two following points will be definitively addressed:

First, the election of the new ESACT Executive Committee, and second, a proposal will be made to modify the constitution. With respect to the election of the new ESACT Executive Committee, I would like to ask the



ESACT members who are interested to become a member of the ESACT Executive Committee, to send his/her application, which has to be seconded by two ESACT members, to Alain Bernard, our secretary. The application should contain some words on the reasons why being a candidate and what he/she would like to push forward during his/her stay on the board (some sort of candidature campaign). In due time the ballot paper together with the final agenda will be distributed to the members, at least 6 weeks ahead of the general assembly of ESACT. In this way, the ESACT members can get an idea of the candidate and can elect the person not because of the name (known or unknown), but also because of his/her ideas with respect to ESACT. On the other side the composition of the ESACT Executive Committee will change because following the constitution the chairman, the secretary, as well as the treasurer have to change. You will hear more on this in the next issue of our newsletters.

With respect to the modification of the constitution, the Executive Committee came to the conclusion that the position of a Vice-chairman should be proposed in order to second the chairman in his duties and replace the chairman for certain tasks. Please, take a look at the article explaining this modification of the constitution. Thus the ballot papers for the election will also ask the ESACT member if he/she agrees with the modification of the constitution.

Other interesting activities during the 19th ESACT Meeting, will be the JIN fair, poster session, traders exhibition and traders reception, and of the famous social events of ESACT: excursion and gala dinner. With the gala dinner the meeting will close and will open the way for Hansjörg Hauser

(GBF, Braunschweig/D) for the organisation of the 20th ESACT Meeting which will take place in Dresden in spring 2007.

You remember that the EU organizes a Marie Curie Programme for increasing the mobility of researchers in Europe. Although our last project proposal which has been prepared together with ACTIP, failed, we think that a new more streamlined and revised proposal for a Research Training Network (RTN) should be prepared and submitted to Brussels. A convenient deadline is the beginning of September 2005 because this leaves enough time for discussing this project end for getting to a version which might be more 'sexy' than the previous one. A RTN project is a mobility 'device' for early stage and confirmed young researchers for short and long term stays in another/foreign lab. I will keep those, who are interested, informed on future activities in this domain.

ESACT is always trying to improve its service for our members. Classical services are the reduced registration fees for the ESACT and JAACT Meetings, the printing of the Proceedings of these meetings, the ESACT web site (www.esact.org), announcement of courses, etc. In addition, we have tried and succeeded to get free access to the electronic versions of CYTOTECHNOLOGY an interesting feature which will be terminated with end of 2004. Very recently we got an offer for free access to the Journal of Pathology. Although this journal is not our first choice, we think that this is a good idea and might be interesting for some of our members. Thus I have asked the journals marketing executive of Wiley and Christophe Losberger to establish such a link for a certain time, which is on the

way.

Finally, some words concerning JIN (Job Information Network). Christophe Losberger has done an excellent work in establishing and improving JIN to the current state. Today's advanced version of JIN allows the rapid and simple advertisement of a new job in industry which can be put onto the website directly by the user (a control is always performed by Christophe in order to avoid any problem with job adverts which are not in accordance with the activities of ESACT). In addition, this website provides also the possibility for young researchers, PhDs, and PostDocs to put their CV for drawing the attention to their 'availability'. This is a very new feature and more than 10 people have already used this feature. The improvement of JIN was relatively expensive and was unfortunately only partly sponsored by CilBiotech (30%). Due to the failure of CilBiotech, ESACT is presently seeking for further sponsorship to cover the bill. The advantages for the sponsors will be advertisement for the company – the company's logo will be integrated into the JIN home page – and, if wished, a direct web link to the company can be installed via the logo. The general advantage for any company putting their open positions on JIN is that JIN works without any fees compared with high advertisement costs newspapers.

Thus I come to the end of my words, and I wish you all the best for Christmas and a successful 2005.

**Otto-Wilhelm Merten, Evry,
16.12.04.**

Constitution Change

From the Executive Committee of ESACT 5th of January, 2005

Objective: Modification of the Constitution.

To the ESACT Member,

*Via this article, the Executive Committee of ESACT communicates a proposal for a modification to the Constitution of ESACT with the following major change: creation of a new officer position in our society's executive committee: the **Vice-Chairman**. The main reason for this major modification is the limited time available for the chairman of the society often leading to the difficulty to find a candidate for the next term of 2 or 2x2 years. This can easily be explained by the constant increase in the work load for everybody working in industry but also in academia. You know our daily life becomes always more stressful, leaving less time for dealing with issues of importance for ESACT. The creation of the Vice-Chairman position will provide the possibility for the Chairman to delegate parts of his tasks to the Vice-Chairman, in addition, the Vice-Chairman is the person who will replace the Chairman for all tasks when the Chairman is busy. In addition, in order to introduce the Chairman to his future task he might pass via the office of Vice-Chairman. For these reasons, the ESACT Executive Committee thinks that the creation of a Vice-Chairmanship is of interest to the society and the society's members.*

During the next General Assembly which will be organized during the ESACT Meeting in Harrogate in June 2005, our members have the right to vote in favour or against this proposed modification.



Dr. Otto-Wilhelm Merten,
Chairman of ESACT
Dr. Alain Bernard,
Secretary of ESACT

There follows a draft version of the modified constitution:

CONSTITUTION OF THE EUROPEAN SOCIETY FOR ANIMAL CELL TECHNOLOGY

1. NAME

The name shall be: European Society for Animal Cell Technology (ESACT).

2. AIMS

2.1 Vision

ESACT promotes the use of animal cells for the benefit of humanity.

2.2 Mission

The aim of the society is to promote the communication of experiences between European and overseas investigators working animal cells to increase their scientific and economic application and to achieve the acceptance of the tools and products derived from them. ESACT encourages interactions between academia, governmental agencies and industry.

2.3 Strategy

ESACT is committed to furthering the use of animal cell technology from the current product areas such as vaccines, monoclonal antibodies, and recombinant proteins into new and emerging fields. These will include gene therapy, tissue engineering, drug and safety testing, and the replacement of animal tests by in vitro systems.

To fulfil this strategy and to draw on the interdisciplinary strengths of our membership ESACT's actions will be directed towards:

1. The organisation of state of the art, scientific meetings
2. The production of a range of publications

3. The support of training activities
4. Interactions with relevant scientific, economic, regulatory and social groups.

3. OFFICERS AND EXECUTIVE COMMITTEE

3.1 The Officers of the Society shall be a Chairman, a Vice-Chairman, a Secretary, a Treasurer and a Meeting Secretary.

3.2 There shall be an Executive Committee of the Society consisting of the officers and four elected members. The quorum at any Committee Meeting shall be five members, including at least two officers.

3.3 The Executive Committee shall prepare the Agenda for meetings of the Society, and between meetings shall act as necessary on behalf of the Society; it shall report on any such actions, as indicated, to the next meeting of the Society.

3.4 The Officers of the Society and the other four members of the Executive Committee shall normally be elected at the Biannual General Meeting (GM). Nominations may be made by the committee or by any two ordinary members and shall be sent together with the written consent of the nominee to the Secretary so as to reach him/her at least two months before the Biannual GM. Such nominations shall be circulated with the notice of that meeting. Members unable to attend the meeting shall be entitled to vote by post. If other nominations are not received for the filling of vacancies, the committee's nominees shall be deemed elected.

3.5 The Chairman, Vice-Chairman, Treasurer and Secretary shall not hold office for more than four consecutive years and the Meetings Secretary for more than two years. They shall not be eligible for re-election to the same office for two years, but they shall be eligible for any other office in the Society.

3.6 The Executive Committees may for special purposes co-opt members to participate in Committee Meetings, but such co-opted members would not be

eligible to vote on resolutions proposed in committee.

4. MEMBERSHIP

4.1 The Society shall consist of Ordinary and Honorary Members engaged in or directing work of the nature indicated in the Aims.

4.2 The names, qualifications, and professional experience of candidates for Ordinary Membership of the Society must be sent to the Secretary on the form provided for this purpose, and the applications approved by the Executive Committee. A list of the newly approved members will be circulated or read out to the membership at the Biannual GM or at an Ordinary Meeting of the Society.

4.3 When a person has been elected a member of the Society, the Secretary shall inform him/her of his/her election and shall send him/her a copy of the constitution and supplementary decisions.

4.4 The Executive Committee shall have the power to terminate a membership if such termination appears to them to be in the interests of the Society. An explanation of such a termination may be requested at the Biannual GM.

4.5 Honorary Members of the Society shall not number more than ten per cent. Nominations for Honorary Membership shall be put forward by the Executive Committee and shall be circulated to members with the Agenda for the Biannual GM. Members who cannot attend the meeting shall be entitled to vote by post. To qualify for election, a nominee must be supported by two thirds of the Members who take part in the voting.

5. FINANCE

5.1 Members shall pay to the Society's account an annual subscription payable in advance, due on 1st January, the amount of such subscription being determined at a Biannual GM of the

Society and continuing in force until changed at a subsequent one. The Committee shall have the power to terminate membership if a member fails to pay his/her subscription after due notice has been given. Honorary Members shall not pay the annual subscription.

5.2 For membership of the Society to become effective, a Banker's Order or cheque for the payment of the subscription shall be received by the Treasurer within three months of the date of election, but the Committee shall have the power to relax this requirement in exceptional cases. Payment by credit cards is equally possible.

5.3 The funds and estates of the Society shall be derived from the annual subscriptions of members, income from meetings, donations, grants and other endowments accepted by the Executive Committee on behalf of the Society. They shall be administered by the Treasurer, acting on instructions given by the Executive Committee or by the Society at its Biannual GM, for the furtherance of the objectives of the Society.

5.4 The accounts of the Society shall be independently scrutinised at the end of each Treasurer's term of office, and a report made by the Treasurer to each Biannual GM.

5.5 In the event of the Society being dissolved for any reason, the surplus funds remaining after satisfaction of debts and liabilities shall not be distributed among the members but shall be paid or transferred to some other charitable institution or institutions having objectives similar to those of the Society and which shall prohibit the distribution of its or their income among its or their members. Such institution or institutions shall be determined by the members of the Society at or before the time of dissolution, and if effect cannot be given to this proviso, the surplus funds shall be devoted to some charitable

objective or objectives.

6. MEETINGS

6.1 The Biannual GM (and, when necessary, an Extraordinary GM) shall be held at a place and time decided by the Committee. The Secretary shall circulate the Agenda to all members at least one month before the meeting. The quorum at any Biannual GM shall be forty ordinary members. Decisions for business items of the Society shall be reached by a simple majority of those voting if not specified otherwise.

6.2 Scientific meetings and symposia relating to the objectives of the Society shall be arranged from time to time by the Committee. One such meeting may immediately precede or follow the Biannual GM and at each Biannual GM the Committee shall submit proposals for the dates of such meetings to be held during the ensuing twelve months. Unauthorised reports of proceedings shall not be disclosed to the press.

7. ALTERATIONS TO RULES

Alterations to any rule of the Society shall be made only at a GM of the Society, provided that notice of such alterations has been given on the Agenda of the meeting and those two-thirds or more of those voting on the alteration signify their assent. Members unable to attend the meeting shall be encouraged to express themselves in letters which will be read out in the meeting. Notice of any proposed alteration, duly seconded, shall be given to the Secretary at least two months before the meeting. No alteration shall be made to the rules which would cause the Society to cease to be a charity at law.

UK Singapore Science Meeting

On the 26th Nov. 2004, His Royal Highness Prince Andrew, Duke of York and Mr. Heng Chee How, Minister of State, Ministry of Trade and Industry launched the UK Singapore partners in science in Singapore. This will be a year long campaign of activities to celebrate the excellence of UK science, engineering and technology and partnership between the UK and Singapore. Among the UK Science Stars who will be taking part will be:-

Dr. Mike Howse, Chief Research & Technology Officer, Rolls Royce.

Prof. Jocelyn Bell Burnell, Discoverer of pulsars, University of Oxford.

Dame Julia Higgins, Professor of Polymer Science, Imperial College.

Prof. Alec Jeffreys, Discoverer of genetic fingerprinting.

Dr. Tim Hunt, Nobel Laureate, Cancer Research UK

For more information, visit www.britain.org.sg
SO

Stem Cell Research at BTI

Human embryonic stem cells (hESC) hold great potential for regenerative medicine because of their ability to differentiate to any cell type in the body. However a major bottleneck is the ability to generate large numbers of hESC for therapeutic purposes. Our group has identified several key areas to focus our research in Figure 1.

These include developing a completely serum free and feeder free culture platform, creating tools and methods for characterizing hESC, which include raising of monoclonal antibodies to novel

Advancing Technologies

Bioprocess Considerations in Cell Therapy

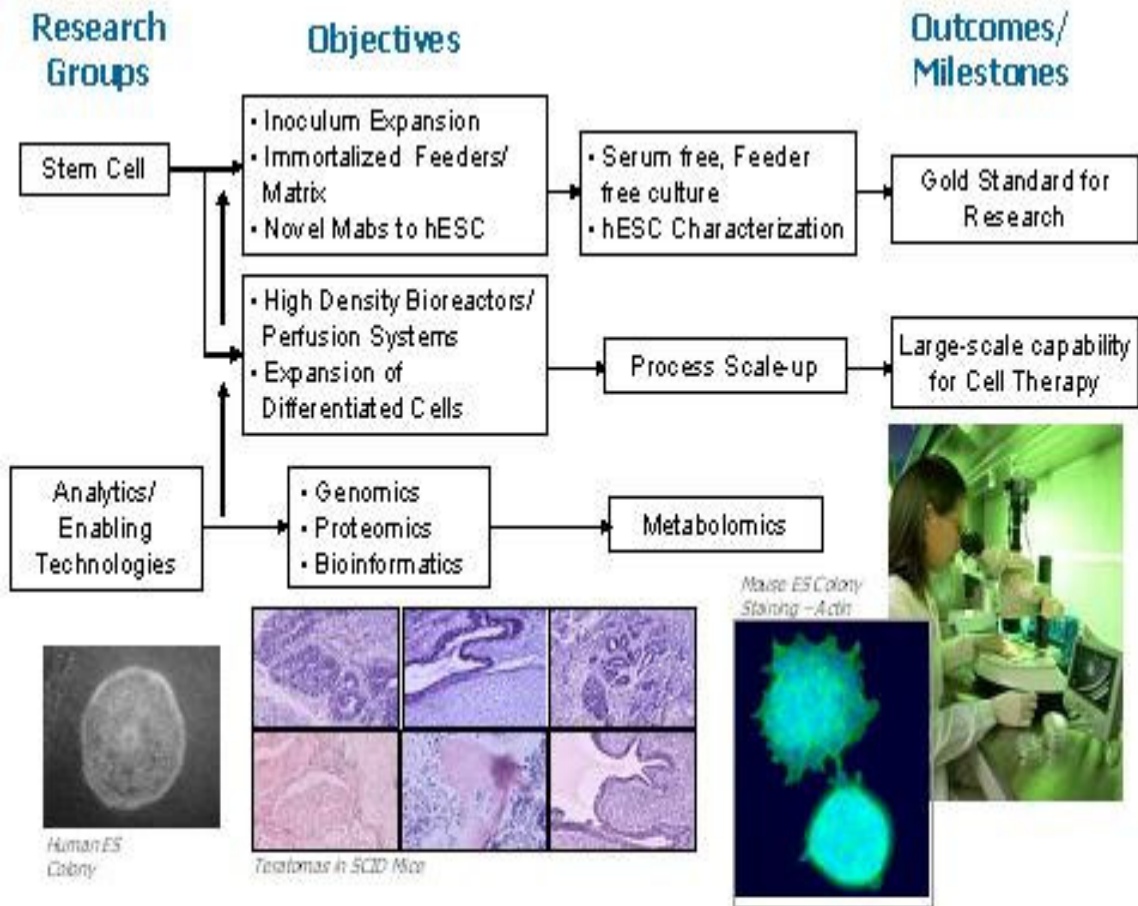


Figure 1: Advancing Technologies – Bioprocess considerations in stem cell therapy

cell surface markers. In addition, we are creating high density cultures of hESC which could later be used in expansion of differentiated progeny. Examples of 2 research themes are given below.

Development of serum free, feeder free culture

Combining the Analytics and Proteomics capabilities of BTI, we are fractionating the condition media produced by feeders using HPLC, antibody arrays, 1 and 2D gel and mass spectrometry to hunt for the active factors that would be able to support hESC culture.

Characterisation of hESC

With the generation of immortal feeders, we are able to scale up hESC and raise antibodies to them. Currently we are characterizing the different targets that these antibodies bind to as potential novel markers of hESC.

Publications:

A Berrill, HL Tan, SC Wuang, WJ Fong, Andre BH Choo, Steve KW Oh, 2004. Assessment of stem cell markers during long-term culture of mouse embryonic stem cells. *Cytotechnology* 44:77-91.

Andre BH Choo, Jayanthi Padmanabhan, Angela Chin, Steve KW Oh, 2004. Expansion of pluripotent human embryonic stem cells on human feeders. *Biotechnol. Bioeng.* 88 (3):321-331.

Steve KW Oh, WJ Fong, YW Teo, HL Tan, J Padmanabhan, A Chin, Andre BH Choo, 2004. High density cultures of embryonic stem cells. Submitted.

Steve KW Oh. Perfusion culture of embryonic stem cells. Patent Application. For more information visit www.bti.a-star.edu.sg

SO

Human Stem Cell Lines for Research and Therapy: A Brief Update

Over the last year stem cell research has continued to progress rapidly and in this brief article I will attempt to outline some of the key developments of particular relevance to the use of human stem cell lines in R&D and therapy.

A notable development in 2004 was the dramatic increase in the number of ES cell lines now derived and available for research. A review late in 2003 estimated a total of approximately 50 ES cell lines available in the world but more recent estimates indicate that this had almost doubled by the end of 2004. The UK Medical Research Council has coordinated a group of international funding bodies to sponsor two international collaborative stem cell projects, one on issues of donor consent in different countries and a second to characterise all the newly available ES cell lines from around the world (see www.mrc.ac.uk). The latter project has now engaged over 15 expert ES cell derivation laboratories and is coordinated by Professor Peter Andrews (University of Sheffield, UK). Each centre will characterise its own cell lines according to an agreed protocol. Flow cytometry data using 15 different ES cell marker antibodies will be submitted from each lab that will also provide samples of undifferentiated and differentiated cultures for RNA expression profiling (targeting 48 genes relevant to stem cells) and for microbiological studies including examination for endogenous retrovirus expression. Identity tests (DNA profile) will be carried out along with tests for genomic imprinting and independent examination of tumours produced by each cell line in studies of pluripotency. The National Institute for Biological Standards and Control (UK) is

acting as the technical “hub” and has been distributing panels of reference antibodies and stable embryonal carcinoma cells for testing in parallel with the local ES cell lines. The UK Stem Cell Bank at NIBSC will coordinate encoding and distribution of ES cell samples to a group of expert analytical laboratories who will report all data to Professor Andrews to be collated as a joint publication later in 2005. At this time the project is anticipated to include data from over 80 ES cell lines.

During 2004 the UK Stem Cell Bank has received accreditation to provide stem cell lines for clinical use from the Medical and Health-care products Regulatory Agency (MHRA) which means that the clinical grade facility for the Bank (built and equipped in compliance with EU GMP) had been designed, constructed and accredited within 17 months of the start of the project. In December 2004 the Bank was also accredited as an International Depository Authority for stem cell lines under the Budapest Treaty (1977) which will be a valuable patent facility for stem cell researchers working on new developments in the use of stem cells. The first two research grade ES cell lines were approved by the Steering Committee for the Bank in May 2004 and the Committee has now considered a further substantial number of new ES cell lines for accession in the Bank during 2005. The approved lines will be made available as safety testing, characterisation, *in vitro* stability studies and depositor qualification of cell banks are completed. The MRC has now also funded a number of ES derivation centres in the UK to develop clinical grade ES cell lines that will pass to the UK Stem Cell Bank and there are similar initiatives elsewhere, and the USA has been particularly active.

Numerous national and international

stem cell networks have been developed during 2004 and whilst I will not attempt to describe these all here, it is hoped that this activity will drive further progress in the science in the coming years. Private funding in the US is driving a powerful lobby to progress work on a broader range of embryonic stem cell lines. Three billion dollars have been committed to this work in California alone, although the outcome of disputes with the federal position has yet to be resolved.

The regulatory environment in Europe has also seen some significant changes in 2004 with the European Human Tissues Directive making progress after a difficult period in which the issues relating to the use of embryonic cells for research and therapy came to the fore. This Directive now seems to be making good progress and should be implemented by 2006. In November 2004 in the UK the Human Tissues Act passed the final stage of Royal Assent and the Human Fertilisation and Embryology Authority (HFEA) has been charged with implementation of the Act and putting in place the codes of practice for clinicians and researchers to address. Notably the Act does not place strict regulation on the use of human cell lines for research which is not the case for the Human Tissues Directive. The legal position for work on embryonic stem cells has been highly variable across Europe. However, appropriate legislation is now in place to permit this work in many European countries and permissive legislation is also now in place in France and Spain.

The clinical application of stem cell lines moves closer to reality. There are now clinical trials finding new uses for bone marrow stem cells and cord blood derived stem cells are fast becoming a standard therapeutic approach. Geron appears to report future clinical trials to use oligodendrocytes derived from ES

cultures. It seems that stem cell therapy may well be set for a major breakthrough in the not too distant future although it will be vital to consider the safety issues carefully ahead of time. There is a need for good quality advice on the safety and standardisation of stem cell therapies and in UK and elsewhere the NIBSC is playing its part in assisting groups to move towards safe and reliable clinical therapies with stem cell lines.

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Nature Biotech Review Paper

Florian Wurm, a member of the XC since 1997 has just published a review paper entitled: “[Production of recombinant proteins therapeutics in cultivated mammalian cells](#)” in the Nov. 2004 issue of Nature Biotech. Here’s an abstract, please see the journal website for the full paper:

Abstract

Cultivated mammalian cells have become the dominant system for the production of recombinant proteins for clinical applications because of their capacity for proper protein folding, assembly and post-translational modification. Thus, the quality and efficacy of a protein can be superior when expressed in mammalian cells versus other hosts such as bacteria, plants and yeast. Recently, the productivity of mammalian cells cultivated in bioreactors has reached the gram per liter range in a number of cases, a more than 100-fold yield improvement over titers seen for similar processes in the mid-1980s. This increase in volumetric productivity has resulted mainly from improvements in media

composition and process control. Opportunities still exist for improving mammalian cell systems through further advancements in production systems as well as through vector and host cell engineering.

Letter from North America

Greetings from North America! As most ESACT members know, on the left side of the Atlantic Ocean there is a series of conferences on Cell Culture Engineering (CCE). Many of us in ESACT attend these to get our 'fix' of scientific discussion and to catch up with transatlantic friends in the off-years from the ESACT general meetings. Originally sponsored by the United Engineering Foundation in America, the conference series has been moved to a successor non-profit organization known as Engineering Conferences International (www.engconfintl.org). As part of this transition, ECI has been steadily reinvigorated under the leadership of Dr. Barry Buckland (Merck, U.S.) and planning for new conferences has proceeded at an unprecedented pace. Of course the CCE series continues in its present format; the tenth conference in the series will be chaired by Professor Jamie Piret at University of British Columbia (jpiret@chml.ubc.ca) and Konstantin Konstantinov of Bayer in Berkeley, CA (konstantin.konstantinov.b@bayer.com). The conference will be held April 23 - 28, 2006 at the Chateau Fairmont Whistler Ski Resort and Spa in Whistler, British Columbia (<http://www.engconfintl.org/6ac.html>), so mark your calendars (too bad for Euro holders that the meeting isn't in the U.S.)! The program is already well-developed, and we can look forward to hearing more about the meeting in Harrogate this spring. We also have plans moving forward for the eleventh meeting, which is tentatively planned for Australia in 2008



with Peter Gray of the Australian Institute for Bioengineering and Nanotechnology, U. Queensland and Carole Heath of Amgen (Seattle) as co-chairs. No jokes about AUSACT!

In addition to the CCE series, ECI is also starting new topical conferences which should keenly interest ESACT members. The closest in proximity is a conference titled "Cell Biology Engineering: The Cell in Context", co-sponsored by the American Society for Cell Biology, and chaired by Prof. Jean Schwarzbauer of Princeton U. and Prof. Linda Griffith of MIT. The conference will be held July 15 - 18, 2005 at U. Washington, Seattle. Program details are available at (<http://www.engconfintl.org/5a1.html>); this conference is a shorter 3-day symposium which will focus on more fundamental cell biological research at the basic science-engineering interface. It looks to be very exciting. Another meeting on the drawing board is "Vaccine and Gene Therapy Technology" to be held in summer, 2006 (<http://www.engconfintl.org/6aa.html>).

Looking forward to seeing all of you soon.

John Aunins

3rd r-Protein production meeting "A comparative view on host physiology"

11th-14th November 2004 Tavira, Portugal

By: T. Battle, H. Hauser, M. Fusseneger, & A Bernard

The conference was organized by Professor Manuel Carrondo and his team from the Instituto de Biologia Experimental e Tecnologica (IBET) Oeiras, Portugal.

1. The conference was split between 6 oral sessions :
2. Regulation of cell growth and product formation
3. Pathways and modulation of folding and secretion
4. Post-translational modifications
5. Cell and metabolic engineering for new and improved protein production
6. Impact of process technology on cell physiology
7. High throughput tools for screening and production

Two poster sessions (83 posters), split as: 33 Bacterial (mainly E.coli), 23 Yeast (mainly Pichia), 10 Mammalian (mainly CHO), 3 Insect/baculo, 15 miscellaneous gene tools (UCOES etc..).

Participation limited to around 254 delegates both from academia and industry.

In his introductory keynote lecture, **Klaus P. Schäfer** (Altana) gave an overview of all expression systems available and indicated that chemical peptide synthesis should be included more and more as a valuable addition to the toolbox of the recombinant protein researcher. 50 aa-long peptides were claimed to be amenable and economically viable via this technology.

Randal J. Kaufman (Howard Hughes Med. Inst., Univ. Michigan) gave an illuminating talk about “factors limiting protein secretion from mammalian cells: the Unfolded Protein Response (UPR)”. He focused on the trafficking of the proteins through the ER, Golgi and trans Golgi network highlighting key loops and regulatory pathways potentially leading to the ER-associated protein degradation (ERAD). He showed data about an eGFP reporter system following the activation of the IRE1 pathway, which proved to be a very efficient tool for monitoring early stage UPR activation. **Pr J. Cole** (Univ. of Birmingham), expert on pathogenic bacteria, presented interesting membrane protein features originating from *Gonococchi* gonorrhoea. He developed explanations about canonical motifs that could help predict membrane protein localization either on the inner or external bacterial membrane/wall. For example in *E coli*: in 95 % of cases if an aspartate is positioned after the LAAC motif, then the protein is going to be routed to the inner membrane. **Dr G. Miksch** (Bielefeld University, Germany) provided a very promising talk on “Libraries of synthetic stationary-phase and stress promoters as a tool for fine tuning of expression and secretion of recombinant proteins in *E coli*.” He described a method for construction, screening and characterization of stationary phase / stress promoters by using libraries of synthetic promoters, which cover a wide range of promoter activity. He highlighted the fact that IPTG induction of T7 promoters may not be ideal due to toxicity and too high Cost of Goods (COGs) for industrial transfer and scale-up purposes.

Ario de Marco (EMBL-Heidelberg) presented a “refined characterization of protein aggregates (including inclusion bodies) forming during strong promoter-induced expression”. A GFP-GST construct expressed in *E coli* was

used as a model. The lysate recovered from bacteria cultured at 20°C was loaded onto a step sucrose gradient and r-protein fractions/aggregated forms were separated. Aggregation index was evaluated based on the 280/340 absorbance ratio, separated by gel filtration, analyzed by electron microscopy and checked for their affinity to the thioflavine T dye. He showed that protein aggregates can be separated in different classes (by using the sucrose gradient) and that the complexity of recombinant protein aggregates is dependent on factors like growth temperature (as expected). **Nico Mertens** provided an excellent talk about “parallel evaluation of expression elements for fast screening of optimal expression strategies in *E coli*”. His conclusions were: 1) To select a mid-power range promoter with no leakiness (e.g. lambda, well repressed), 2) To select cautiously the translation initiation region + ribosome binding sites. When wrongly chosen, it could make a soluble protein to be expressed as insoluble whereas, if optimal system, some proteins became 1,000 times more soluble. He claimed that a bacterial protein could be declared as soluble only based on gel filtration analysis. **Ian Hodgson** (Avecia, UK) described their proprietary bacterial expression system named “p-Pop”. This system is based on a “palindromic operator plasmid” inducing a conformational change of the repressor allowing a very tight repression in the absence of IPTG. They claimed a far better repression in the absence of IPTG linked to a very fine-tuning of IPTG titration during induction. This apparently allowed better conditions for protein secretion without inclusion bodies formation. A process implicating inclusion body formation and a typical low 15% DSP recovery and renaturation yields, is probably more favourable if switched to generation of soluble proteins at the cost of lower expression levels (to avoid

denaturation and re-folding).

Stefan Wildt (GlycoFi, USA) presented an overview of the capabilities of the *Pichia pastoris* system in which they have totally re-engineered a human-like glycosylation machinery after having knocked out competing yeast glycosylation pathways. The main advantages of this fairly recent technology are: better control of glycosylation and more uniformity of the final product (potentially a single glycoform), speed to derive a production line (a couple of weeks) and productivity (g/l range).

Dana Andersen (Genentech, US) described the numerous improvements they could bring to a process designed to express antibody fragments in *E. coli* periplasmic space. They identified some bottlenecks in the final assembly of the two chains as being the availability of the heavy chain and its “competency” to assemble. These bottlenecks limited the expression level to 1.5 g/l, whilst 14 g/l of various antibody related species were also present (i.e., about 10% of the two chain are assembling into an intact Fab fragment). The availability of the heavy chain was improved by separating the expression of the two chains under different promoters and timing the induction of the heavy chain earlier than that of the light chain. This led to an increase in overall expression level to 4 g/l. The “competency” of the heavy chain to assemble then remained the only bottleneck and it seemed that some of it remained insoluble. **Anne B. Tolstrup** (Symphogen A/S, Denmark) reported on the “production of recombinant human polyclonal antibodies against rhesus-D in CHO FLP-in cells”. The product currently under development (Symphoglobulin-D) is a recombinant human polyclonal anti-rhesus D to be used for the prevention of hemolytic disease in the newborn. They are pursuing a strategy based on site-specific integration, e.g. using the FRT/

Flp-in recombinase system. This aims at integrating plasmids encoding the antibodies at the same position in each of the transfected cells (i.e. without rounds of clonal selection). Also, only one plasmid will be integrated in each cell, thus eliminating the risk of heavy and light chain gene repair. Further, the genetic construct is made so that each cell expresses the same pre-chosen constant region gene, meaning that the expressed antibody is monoclonal with respect to the majority of the coding sequence (all the molecule except for the V region sequence). Together, these features increase the likelihood that such polyclonal antibody producer CHO cell lines will display sufficiently similar characteristics with respect to productivity and genetic stability to enable a controlled GMP production of recombinant polyclonal antibodies.

Nigel Jenkins (Serono) insisted on the fact that post-translational modifications are not only restricted to glycosylation and that other key modifications take place in the ER/Golgi, i.e. de-amidation, oxidation, proteolytic cleavage. Extra chaperoning in CHO retard protein synthesis and drastically prolongs cell population doubling time!! **Thierry Battle** (Serono) described protein expression and purification/QC within a functional genomics discovery pipeline. He reviewed the positioning of three expression systems in the context and outlined the high level of productivity obtained as measured by the number of genes expressed and proteins purified for functional analysis. **Dr Raj Haldankar** (Amgen) presented Amgen’s vision about mammalian high throughput expression: by “large-scale transient transfection”. Their favorite vector/host combinations for transient expression were: Hek293 T in pCDNA3.1 with SV 40 promoter, Hek293EBNA in pEAK vectors with CMV promoter, COS-PKB in pAWA vectors

with SV40 promoter, CHO-S in pEAK vectors (or pCDNA3.1) with CMV promoters. Their CHO-s transfection system was made serum-free in CHO-CD/DMEM F12 medium mixture. They insisted on the importance to co-develop the transfection system in parallel with medium optimization as they boosted secretion in a transient mode due to the medium. They claimed that they could almost double the seed culture concentration (around 4 to 5 million cells/ml (helping them to transfect numerous cultures while maintaining a limited number of spinners).

Hitto Kaufman (BI pharma, Germany) illustrated a concept of fast-track cell line development for monoclonal production processes. They usually are able to deliver a production cell line in 9 months and then need an additional 3-4 months to reach an optimized process with 1-2 g/l titres. He explained that they normally screen their clones in the same basal medium that will be used in the large scale bioreactors. Starting from a proprietary, engineered CHO host overexpressing bcl-xl (anti-apoptotic gene) they improved selection by using a neomycin resistance gene mutated to have lower specific activity. **Nicolas Mermod** (EPF Lausanne) presented the background and applications of MAR elements, also called SAR or S/MAR. These chromosomal sequences have been isolated from chicken and mammalian species and support various activities of colocalized expression cassettes in mammalian cell lines. In particular, a stimulation of expression strength can be routinely achieved. **Hansjörg Hauser** (GBF, Braunschweig) presented the establishment of mouse cell lines by the introduction of immortalizing genes into primary cultures of embryonic and adult animals and an embryonal stem cell line. Fibroblastoid and endothelial cell lines that specifically expressed endothelial

markers were created. The properties of the resulting cell lines were reproducible, independent of the donor mouse strain and showed no oncogenicity. The presented work suggests that conditionally immortalized cell lines have advantageous properties over constitutively immortalized cells.

Battle, Hauser, Fusseneger, Bernard

Future Meetings

- Process Validation for Biological Production Conference

14-15 February 2005, Basel, Switzerland

Information: www.ef-international.co.uk

- World Life Sciences Forum, BioVision

April 11-15, 2005, Lyon, France

Information: www.biovision.org

- Molecular Medicine Tri-Conference

April 19-22, San Francisco, USA

Information:

www.chimolecularmed.com

- Pathway Analysis for target and compound evaluation

April 20-22, 2005, San Francisco, USA

Information:

www.chimolecularmed.com

- 7th Conference Protein Expression in Animal Cell

Crete, Greece

September 18 - 22, 2005

Information: www.peace-conference.org

Joke Corner

Many years ago, in Scotland, a new game was invented. It was ruled this game was for **Gentlemen Only...Ladies Forbidden**"...and thus the word **GOLF** entered into the English language.

The first couple to be shown in bed together on prime time TV were Fred and Wilma Flintstone.

Every day more money is printed for Monopoly than the US Treasury.

Men can read smaller print than women can; women can hear better.

Coca-Cola was originally green.

It is impossible to lick your elbow.

The percentage of Africa that is wilderness: 28% (now get this...)
The percentage of North America that is wilderness: 38%

Each king in a deck of playing cards represents a great king from history:

Spades - King David
Hearts - Charlemagne
Clubs - Alexander, the Great
Diamonds - Julius Caesar

111,111,111 x 111,111,111 =
12,345,678,987,654,321

Improving English

Having chosen English as the preferred language in the EEC (now officially the European Union, or EU), the European Parliament has commissioned a feasibility study in ways of improving efficiency in communications between Government departments. European officials have often pointed out that English spelling is unnecessarily difficult; for example: cough, plough, rough, through and thorough. What is clearly

needed is a phased programme of changes to iron out these anomalies. The programme would be administered by a committee staffed at top level by participating nations.

In the first year, for example, the committee would suggest using 's' instead of the soft 'c'. Certainly, sivil servants in all sities would resieve this news with joy. Then the hard 'c' could be replaced by 'k' sinse both letters are pronounsed alike. Not only would this klear up konfursion in the minds of klerikal workers, but typewriters kould be made with one less letter.

There would be growing enthusiasm when in the sekond year, it was anounced that the troublesome 'ph' would henseforth be written 'f'. This would make words like 'fotograf' twenty persent shorter in print.

In the third year, publik akseptanse of the new spelling kan be expekted to reach the stage where more komplikated changes are possible. Governments would enkourage the removal of double letters which have always been a deterrent to akurate speling.

We would al agre that the horrible mes of silent 'e's in the languag is disgrasful. Therefor we kould drop thes and kontinu to read and writ as though nothing had hapend. By this tim it would be four years sins the skem began and peopl would be reseptive to steps sutsh as replasing 'th' by 'z'. Perhaps zen ze funktion of 'w' kould be taken on by 'v', vitsh is, after al, half a 'w'.

Shortly after zis, ze unesesary 'o' kould be dropd from words kontaining 'ou'. Similar arguments vud of kors be aplid to ozer kombinations of leters.

Kontinuing zis proses yer after yer, ve vud eventuli hav a reli sensibl riten styl. After tventi yers zer vud be no mor trubls, difikultis and evrivun vud fin it ezi tu understand ech ozer. Ze dremms of the Guvermnt vud finali hav kum tru.

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