European Society for Animal Cell Technology

Newsletter

April 2005

Editorial

Dear Readers,

After a short Easter break, I suspect that many of you are preparing to attend the next ESACT meeting in Harrogate. In this issue, our Chairman brings us up to speed on the latest developments and there are 2 conference reports from JAACT and ACS. Alain highlights the upcoming Executive Committee elections with nominated candidates.

Following which we have a short piece on the state of human embryonic stem cell maintenance from me, an update from Merlin about a New Stem Cell Institute and the Biomanufacturing Training Centre in Ireland and an article from Dr. Stephen Taylor, Managing Director of Avecia on the challenges of the Bioprocess Industry. Finally, we welcome new members to our society.

Looking forward to re-acquaintance with old friends and meeting new ones in June!

Chief Editor, Steve Oh

P.S. I hope all members have received their ESACT Proceedings from Granada, as I just have. Thanks Quico!

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A Word from the Chairman

Dear ESACT Member,

My time as ESACT chairman is almost over and from the next ESACT meeting in Harrogate in June onwards, Florian Wurm will represent ESACT for the upcoming two to four years.

Thus I'd like to take a look back at what has happened during the last years and the advances which have been achieved. As indicated by our previous chairman Manuel Carrondo four years ago (ESACT Newsletters April 2001), animal cell technology is gaining more and more importance, is continuously growing and new products are coming to the market. Besides vaccines and recombinant proteins, in particular, monoclonal antibodies (Mabs) have become 'our' products. The increasing economic interest for Mabs and thus the need for large scale production plants are shown by actual and prospected future sales. 17 existing Mabs produced for human therapy generated sales of US$ 6.5 billion in 2004 and 16 new antibody products will increase these sales by about 3 billion US$ in 2008 (Reichert & Pavlou (2004) Nat. Rev. 3, 383).

These perspectives demonstrate the increasing importance of animal cell technology, but also the need for investment in manufacturing plants and for optimising the engineering of these large scale plants. On the other side, the development and optimisation of new producer cell lines (cell engineering) with improved characteristics in large scale production reactors is ongoing and the use of such cell lines under optimised culture conditions allows yields of several grams of recombinant protein per litre today. The 10 g/L yield which was an utopic objective 10 to 15 years ago seems to be reachable today.

Looking to the future, more patient destined therapies will be available in such new domains as tissue engineering and cell/gene therapy in a short or medium time frame. Although large scale manufacturing will not be the choice for these approaches, new sophisticated small scale, patient specific cell production systems will be of high importance and their development are on the way. Presently this is a fast developing field and new approaches based on stem cell technology (embryonic and adult) will give it a further push.

These positive developments demand specialists in all job categories in industry, from technician up to scientist, and we have tried to draw the attention of the Commission in Brussels to the increased needs for specialists whose education and training take time and are rather costly. However, this turned out to be almost in vain. Despite the joint efforts together with ACTIP (Expression of Interest and submission of a project in the frame of the Marie Curie Programme ("ACTraining")) we failed to get to a project in this field, although it seems that some people from the Commission are aware of the need for supporting more training efforts in animal cell technology and also the danger that industry will invest only where the specialists are 'produced' and are available. Thus , we are discussing with them on the submission of a new Marie Curie project in the field of animal cell technology. In any case, it is hoped that the situation will improve with the 7th framework programme. And it seems that the commission is planning to move the emphasis of this new programme partly to more applied research. The only positive point which can be mentioned here is the fact that for many years ESACT alone or together with other organisations provided bursaries for young students and cell culture scientists to participate at ESACT meetings.

This is a tradition that has proven to be very successful.

In the context of ESACT's service to its members and the industry active in animal cell technology, ESACT has improved the features of JIN (the Job Information Network) and it is now much easier to advertise new jobs and also to put a job search on JIN (a new feature), thanks to Christophe Losberger, our web manager.

ESACT was and is the European Society for Animal Cell Technology and in the past only European residents were full members with all rights. The constitution was modified in the sense that there is no difference any more between European and extra-European members. This opening was necessary because Europe is not the only place where animal cell technology is used, but other countries in the world (in particular North America) are more important in the animal cell technology at least with respect to the overall activities. Thus, there was a need to completely integrate the extra-European specialists, engineers, and scientists active in animal cell technology into ESACT and provide them with the same rights as the European residents. A positive effect was directly observed during the last ESACT Meeting (the Granada Meeting in 2003) where the number of the North American participants increased significantly compared to previous meetings.

What will the future bring? It is clear that we do not know this; however, one thing is sure that ESACT will have a new executive committee with experienced ESACT committee members, and most of the officers (Florian Wurm and Alain Bernard) have been in charge of the organisation of an ESACT meeting in the past. The officers together with the elected members will run the society for the next 2-4 years and will further the service to our members. So, I would like to thank everybody who helped in running the society during the last four years and I wish all colleagues and ESACT members all the best for the future and good luck for the new committee!

Otto-Wilhelm Merten

Report on the 17th JAACT Meeting

"Industrial Applications of Animal Cell Technology"

Prepared by Thomas Noll, Pablo Umana and Francesc Gòdia for ESACT

The 17th Annual and International Meeting of the Japanese Association for Animal Cell Technology, JAACT 2004, was held in Nagoya, from November 15th to 18th in Nagoya. The meeting was chaired by Prof. S. Iijima, and gathered about 200 delegates from Japan and about 70 from countries abroad Japan. The main programme was organised in eight symposia, with 40 oral presentations and 3 plenary lectures. In addition, five workshops with shorter oral presentations were also organised, in a parallel session to the Symposia, and more than 80 contributions were discussed in the poster format, with specific short presentations and poster discussion session. The programme also included luncheon seminars, where lunch was combined with presentations from companies. On the whole, it was a very intensive and complete meting. The complete programme was developed in three days, in a very tight schedule and efficiently organised. ESACT contributed to the programme by organising a specific ESACT Lecture, consisting in three oral communications from T. Noll (Jülich, Germany), P. Umana (Zürich, Switzerland) and F. Gòdia (Barcelona, Spain). In addition, other ESACT members contributed oral presentations. In this report, the main

trends of the sessions are summarised.

Report on the 17th JAACT Meeting

Murakami Memorial Lecture

This special lecture was presented as a memory to Prof. Hiroki Murakami who passed away 4 years ago. He was the founder of Animal Cell Technology activities in Japan, and JAACT. In the JAACT meeting series, he was a key person in introducing science and ACT among young researchers especially in using English as the language for the meetings, as a clear sign for the need for international communication in Science.

The lecture was given by Prof. Gordon H. Sato, director of the Manzanar Project in US. Prof. Sato discussed some of the highlights of his long scientific career in US, where he was born, like the discussions on selection versus differentiation in the evolution of cells in culture or the development of defined media. He made special emphasis on his views on the importance of tissue engineering as the science for the future, and its implications in many fields, such as endocrinology and oncology, in the way that experimentation with cells in culture is providing tools for the understanding at the level of the tissue and animal physiology.

Symposium 1. Recent development in the small RNA world

A complete symposium of the meeting was dedicated to this recent breakthrough in the biological sciences, including a very comprehensive plenary presentation by Prof. K. Taira, presenting the generation of libraries of hammerhead ribozymes with randomised binding arms and/or of U6 and t-RNA-driven siRNAs. The transfection of cells with these libraries was shown to be a powerful tool to identify relevant genes involved in important cellular processes such as apoptosis, cancer metastasis, and cell differentiation pathways. The session also included presentation on the methodology to introduce RNA-i in *C. elegans*, and the connection between RNA-i and the fragile X syndrome. Finally, a presentation on the complex RNA-i patent situation was also given, with analysis of some specific cases.

Symposium 2. Stem Cells Technology

The second symposium of the meeting was also dedicated to a very relevant topic, such as stem cells technology, and also included a Plenary Lecture, that was given by Prof. C. S. Pottem on a very detailed description of the adult epithelial stem cells, particularly those present in the intestine, discussing their number, characteristics and genome protective mechanisms, particularly those involved in the protection against the risk of replication induced errors. The rest of the session had contributions on the generation of neural stem cells genetically modified for brain repair, the study of adhesion on microcarriers of mesenchymal stem cells, and the development of low-serum culture for the expansion of stem cell populations.

Symposium 3. Food Allergies – Current Perspectives

The topic of food allergies has received a lot of interest in the JAACT meeting, with five

oral presentations. They were focussed on two main topics. On one side, the development of new methodologies for the detection of allergenic substances in processed food, particularly based on ELISA concepts was discussed. The role of animal cell technology as a supporting technology for the developments of these tests was made evident. On the other side, the discussion was concentrated in the legal and regulatory developments around this topic, particularly with implications on food labelling.

Symposium 4. Advanced functional water for the prevention of diseases

A specific and innovative session, with three oral contributions, was dedicated to the concept of treated water as a vector for enhancing population health. It is the observation made at the physiological level that originates for such water treatments. Specific examples of this approach were given by the positive effects demonstrated in a clinical trial on the use of potable electrolysed alkaline water on the prevention of gastrointestinal symptoms. Electrolysed reduced water also received attention regarding its effects in the reduction of oxidative stress, particularly that induced by haemodialysis in final-stage renal disease. Also its effects on the reduction of oxidative species such as active oxygen donor were discussed.

Plenary Lecture. Prof. N. Hirokawa.

Prof. Hirokawa plenary lecture was one of the highlights of this JAACT meeting. In a very educational presentation, he discussed on intracellular transport and molecular motors, KIFs structure, dynamics function and diseases, summarizing a vast research effort carried out by his team at the University of Tokyo. Using advanced tools, the characteristics and mechanisms of the role of kinesine superfamily proteins, KIFs, in the transport of functional molecules in neuron cells was elucidated and its relationship with various cell functions and fundamental development events were presented. Finally, the identification the mechanisms of how these motor proteins KIFs move along the microtubes of the cells was elucidated using molecular biophysics, cryoelectron microscopy, X-ray crystallography and optical trapping nanometry.

Workshop on Cell culture engineering and production of biologicals

This workshop was celebrated partially in parallel to the main sessions, in a format of short oral presentations, covering contributions from different groups in the more mature areas of cell culture technology. It combined presentations from academia and industry covering a broad spectrum of topics ranging from cell line development/ engineering, functional genomics and medium development up to primary cell culture.

Duk Jae Oh from the Sejong University, Korea, described the development and use of a depth filter technology for long-term perfusion culture of antibody producing CHO cells. Cell densities up to $3*10^7$ mL^{-1} were maintained for up to 1800 h. The antibody productivity was more than 70fold higher than that of a simple batch fermentation.

Danny Wong from the Bioprocessing Technology Institute in Singapore presented interesting results on the transcriptional investigation of apoptotic events in CHO batch and fed-batch fermentations using their in-house developed CHO cDNA array. Time-dependent upregulation of early and late pro-apoptotic effector genes could be observed. While receptor-mediated apoptosis induction is mainly mediated by Fas, FADD

and Daxx, the mitochondrial-mediated apoptosis is dominated by members of the Bcl-2 family (Bad, Bax, Bid). Several other genes also appear to be upregulated during apoptosis induction but their exact role still has to be elucidated.

Philip Sass (COO of Morphotek, Pennsylvania) gave a keynote lecture of the company's Morphogenics technology as a tool for drug discovery. Several examples were presented showing impressive improvements in cell specific productivity or antibody binding affinity. From a completely different topic Akira Mori, university of Tokyo, described the development of a migration assay to investigate the intestinal epithelial cells and immune cells.

Philip Offin, (The Automation Partnership) presented the company's SelecT system, an automated cell culture processing system for robotic handling of up to 400 tissue flasks which was developed in collaboration with major pharmaceutical companies. In the discussion Dr Offin mentioned that a system for automated handling under controlled conditions is under construction.

Satoshi Terada, (University of Fukui) suggested sericin, a major component of raw silk, as cell culture supplement for serum replacement with several potential applications from cryoprotection to growth and production enhancement. The results being presented were comparable to an FBS containing control culture but a comparison to state-of-the-art chemically defined culture media or media containing hydrolysates was missing. Furthermore it remains unclear whether there are batch to batch variations in the sericin. Alistair Irvine (ML laboratories, UK) presented an interesting approach for the generation of high production cell lines in a short time. When linking the transgene to UCOEs (ubiquitous chromatin opening elements) a larger number of transgene expressing clones showing higher and more stable expression can be generated compared to transgenes not being linked to UCOEs. The production of 1g of antibody from 10 liters of cell culture within about three weeks after transfection was described.

Symposium 5. Metabolomics as a powerful tool in post-genome era.

Within this symposium six presentations were given and the term metabolomics is still being used very differently. The papers covered the identification and quantification of cellular peptides, lipids and proteins as well as metabolites from the central metabolism. In the discussions another problem in animal cell metabolomics became obvious. There is still no reliable technology available for rapid quenching of the cells' metabolism, making the quantification of intracellular pools with a rapid turnover very difficult. Strong improvements have been made regarding the identification and quantification of metabolites using LC-MS and MS/MS techniques. The presentations covered quite diverse topics from the effect of broccoli on oxidative stress, inflammation and hypertension (Dayan Goodenowe, Phenomenome Discoveries Inc), the establishment of cellular peptide databases (Naoto Minamino, Osaka) and metabolic flux analysis in plant cell culture (Ei-ichiro Fukusaki, Osaka) up to comparative evaluation of lipids, proteins or central metabolites in various tissues and diseases (Hiromasa Toja, Osaka; Toshihide Nishimura, Tokyo and Chris Beecher, Metabolon).

Symposium 7. Process Development and Manufacturing of Biologicals

Highlights of this Symposium on Process Development and Manufacturing of Biologicals were the characterization of glyco-engineered therapeutic anti-cancer antibodies and their manufacturing process, as well as the continued development of extremely high-yielding fed-batch processes based on CHO and NSO recombinant cell lines generated with glutamine synthase (GS) selection.

Researchers from Kyowa Hakko described the generation of a fucosyltransferase-knock-out CHO cell line derived from dhfr- CHO-DG44 cells. The cells were engineered for the production of therapeutic IgG1 antibodies lacking fucose residues alpha 1,6-linked to the core of the oligosaccharides attached at the conserved Asn 297 glycosylation site of IgGs. Such glycoengineered antibodies show large increases in biological activity (immune effector cell-mediated killing of antibody-targeted cells). The basis for the increased biological activity was also characterized and presented in this symposium by Kyowa Hakko researchers, who showed a large increase in binding affinity of the engineered antibodies to antibody Fc-receptors found on immune system cells such as NK cells and macrophages.

Shinobu Kuwae from Mitsubishi Pharma presented a high-yielding fed-batch process for the production of recombinant human antithrombin (AT). Use of recombinant cell line generated with Lonza's CHO-GS expression system led to product titres above 1g/l in serum-free medium, allowing the researchers to conclude that this process can now compete economically with AT production from human plasma. Yet another example of the usefulness of the GS selection system for rapidly yielding very productive cell lines was presented by Amid Varma of MedImmune, who has developed a serum-free fed-batch process using an NSO-GS-cell line for the production of a recombinant human therapeutic antibody. The process reaches antibody titres of 5g/l. The researchers suggest though taking a close look at the intellectual property landscape governing commercial use of the NSO system for recombinant antibody production.

Conference Report: 229th ACS National Meeting, San Diego
Division of Biochemical Technology, March 13-17, 2005
Prepared by: Niki Wong, Bioprocessing Technology Institute

Upstream

BIOT 100 - Tuning eukaryotic cell signals with RNAi for increased product synthesis
John March (University of Maryland) reported the use of double stranded RNA (ds RNA) to knock down the expression of Dpten, TSC1 and Archipelago, genes involved in the insulin signaling pathway and cell cycling in insect (Drosophila) cells. He postulated a reduction in cell proliferation or cell growth arrest on knocking down these genes. He mentioned that serum deprived insect cells had the ability to take in dsRNA readily upon re-addition of the serum into the culture. This was an interesting point which seemingly only applied to insect cells though he did not further elaborate on it. However, he did mention that insulin could also replace FBS to exert this effect. RNAi using dsRNA resulted in varying degrees of knockdown for each of the genes, and also subsequent increase in product yields.

BTEC 20 - Genomics approach to metabolic engineering of glycosylation

Mike Betenbaugh (John Hopkins University) reported the continuation of his work on insect glycosylation. In this talk, he reported the cloning of insect homologs of the sialic acid synthase and CMP-sialic acid synthase. In both cases, the insect homologs were cloned using PCR amplification where primers were designed from the human homolog of the genes. He reported that trace amounts of sialic acid synthase was detected in various tissues at different stages of insect development but not in insect cell lines. As for CMP-sialic acid synthase, the expression seemed to vary across the different stages of development in the tissues (regulated during development) and again no expression was detected in the cell lines. Both these findings demonstrated the need for recombinant expression of these genes in insect cells. Another interesting observation was how CMP-sialic acid synthase in insect cells seem to localize in the Golgi instead of the nucleus as with human CMP-sialic acid synthase. These had implications on the mechanisms that insect cells adopt for sialic acid synthesis.

BIOT 392 - Engineering mammalian cells by altering epigenetic gene silencing

Gargi Seth (University of Minnesota) reported her findings on the study of NS0 cells that were adapted to cholesterol free media (NS0-r). When she considered the transcript levels of representative genes in the cholesterol biosynthesis pathway, she found that Hsd17b7 was found 15 fold upregulated in the NS0-r as compared to original NS0 cells. She postulated that the regulation of Hsd17b7 could be through DNA methylation and verified this through methylation specific PCR analysis. She used the de-methylating agent 5-azacytidine to create NS0 single cell clones that could grow in cholesterol free media, where some isolated clones were found to be have 100 fold increase in Hsd17b7. She verified that these clones had de-methylated Hsd17b7 and reported that she managed to reduce the reversion frequency of NS0-cholesterol independent clones to cholesterol dependent clones from 10^{-6} to 10^{-3}.

Transcript expression studies

BIOT 218 - Butyrate impacts genes associated with erythropoietin glycosylation

Christopher Crowell (University of Colorado Health Sciences Center) presented results from a microarray analysis that studied the effects of sodium butyrate treatment on a fibrosarcoma cell line (HT1080) producing EPO. Two millimolar sodium butyrate was used in his experiments and this led to a 4 fold increase in specific productivity. An increase in polylactosamine N-glycans and a decrease in O-linked site occupancy and sialylation was reported based on glycosylation anlaysis of the EPO product. An Affymetrix chip was used with 327 genes linked to glycosylation available on the chip. Twenty-six genes were significantly up or down-regulated in the analysis. It was felt that the interpretation of microarray results was not coherent. The main finding discussed was a decrease in CMP-sialic acid synthase expression with a conflicting increase in intracellular CMP-sialic acid pool (measured using intracellular sugar nucleotide analysis). Decrease in CMP-sialic acid synthase expression would lead to decreased synthesis of CMP-sialic acid. On the other hand, an increase in expression of lysosomal sialidase and lysosomal sialic acid transporter expression was also measured in the microarray analysis and this could increase the sialic acid pool through the salvaging of sialic acid from existing sialoglycoproteins. The effect of the 2 conflicting pathways still resulted in an overall increase in CMP-sialic acid pool.

Modeling

BIOT 159 - Modeling microheterogeneity of protein N-glycosylation
Patrick Hoossler (University of Minnesota) reported the development of a mathematical model for glycosylation. It involved the glycosylation reactions in the Golgi which was split into the cis, medial, trans-Golgi as well as TGN compartments. These compartments were thus considered as reactors in series and reactions were modeled using random bi-bi kinetics. It involved both glycan and nucleotide sugar mass balances that consisted of both reaction and transport terms. Using a single compartment and considering it as a batch reaction, he demonstrated as a proof of concept that the model worked since it predicted correctly that glycosylation after 10 minutes was not as complete as glycosylation after 80 minutes. Following that, it was considered as continuous mode which could allow for a residence time distribution of the glycans in the various compartments. This accounts for incompletely processed glycans. Finally, the 4 compartments were modeled together where 4 reactors in series resembled plug flow. Data which predicted the various types of glycan structures under these various conditions was presented.

BIOT 216 - Mathematical modeling of nutrient perturbations in cell culture
Ziomara Gerdtzen (University of Minnesota) constructed a kinetic model of cell metabolism and 3 key processes were identified as possibly limiting: glucose transport into the cytosol, lactate dehydrogenase reaction and pyruvate transport into the mitochondria. As these were regulated through transcriptional activation, it explained why metabolic shift in cultures would take as long as 72 hours to occur in cell cultures.

Presentations from industry

BIOT 82-Rapid production of proteins in large-scale transient transfections
Dorothea Reilly (Genentech) reported the development of transient transfection procedures in 1 to 100L scale suspension cultures. She reported that optimization involved factors such as type of transfection reagent (DMRIE-C was the chosen transfection reagent), DNA transfection conditions e.g. DNA to reagent ratios, and culture conditions at which transient transfections took place. She gave the impression that transient tranfection technology was increasingly being used in industry to produce compounds in sufficient quantity for initial testing, even at the screening stage, where several potential candidates would be produced and used for further testing, to assist in the evaluation of potential targets for therapeutic uses. She also showed data that glycosylation profiles remained consistent for proteins produced through transient transfection versus stable cell lines. However, titers for various transfection attempts across different culture scales were rather variable and did not seem to be well controlled. She also mentioned that whilst transient transfection was being used, it never completely replaced the generation of stable cell lines. Transient transfection just served to expedite the generation of protein product required for testing, given the much longer time required to generate product from stable cell lines.

BIOT 124-Gene function discovery in metabolic engineering
Tina van Dyk (Dupont) reported the use of genome wide tools like DNA arrays and reporter gene arrays (Lux arrays) to derive function of unknown genes in the metabolic

pathway of *E.coli* cells. In particular, the Lux array consists of a sampling of 33 % of E. coli transcriptional units where the promoter region of different genes was fused to reporter genes (luciferase) and used to probe for transcriptional upregulation in response to stress conditions. Through this analysis, they reported the discovery of a novel efflux transporter, verified the function of this transporter through mutant studies and subsequently found the substrate to the transporter using a screen involving a chemical library of compounds. The use of the Lux array seemed rather interesting as a screen for potential upregulated genes (Van Dyk, PNAS, 2001)

BIOT 189 - Interactions of cell culture medium components with polymer surfaces

Gina Altaras (Merck) reported a study on the extent at which media components e.g fatty acids, vitamins, cholesterol bound to polymer surfaces. This study was motivated by an observation that composition of media components was not constant as it was stored in bottles and bags. The main example cited was linoleic acid. Through small scale radioactive studies, 90 % of linoleic acid was found to bind to polyethelene surfaces within 6 hours. Another observation was how cholesterol with cyclodextrin carrier bound much faster to polymer surfaces as compared to cholesterol alone. The recommended solution was to add these components just before use through the preparation of 100x stock. It was found that stock dissolved in ethanol had improved recovery during pre-filtration.

BIOT 15 - An economic cell-free protein synthesis system

Kara Calhoun (Stanford University) reported the development of a cell-free system which utilizes glucose instead of PEP and nucleotide monophosphates (NMP) instead of nucleotide triphosphates (NTP). This could be carried out by changing reaction conditions to mimic the cytoplasmic environment more closely. This resulted in increased yield and lower cost of reagents. The limitation was the ability of these cell-free systems to perform the more complex post-translational modifications like glycosylation.

BIOT 24 - Engineering the next generation of therapeutic proteins

S Cho (Ambrx, San Diego) reported the development of an orthogonal suppression system in *E. coli* that pairs novel chemically specified amino acids with mutant forms of tyrosyl tRNA synthetases. This allows newly defined amino acids (beyond the 20 natural amino acids) to be added onto synthesized proteins. One example given was an amino acid with a PEG conjugate. The addition of PEG preserved potency and enhanced pharmacological performance and even allowed the formation of homodimers through PEG conjugation.

BIOT 81 - High-throughput screening for antibody process development

Robert Balcarcel (Vanderbilt University) reported the development of a high throughput assay for screening applications such as evaluating cell lines, testing the effects of media formulation and media additives. This is definitely an assay that seems easy enough to adopt if we had a scientific problem we wanted to analyze that involved screening of many conditions in cell culture. In particular, he focused on the study of monoclonal antibody (Mab) enhancers e.g. DMSO, NaCl, sodium butyrate and rapamycin. The parameters that he measured using this assay included cell viability (Alamar blue reaction), glucose, lactate and Mab production. But the findings did not show significant increase in specific productivity from the addition of these compounds, where only rapamycin was reported to have statistically higher specific productivities.

ESACT Executive Committee Election & General Assembly of ESACT—Harrogate, 7th June 2005

The next elections to the ESACT Executive Committee and General Assembly of ESACT will take place, as usual, during the ESACT General Meeting in Harrogate, UK in June. This bi-annual General Assembly of ESACT will take place during lunch time on the 7th of June 2005.

Following our call for nominations, we received the following proposals

Name (by alphabetical order)	Affiliation
Paula ALVES	Instituto de Tecnologia Química e Biológica, Portugal
Mohamed AL-RUBEAI	University College Dublin, Ireland
Thierry BATTLE	Serono, Switzerland
John BONHAM-CARTER	Magellan Instruments, UK
John DAVIS	Bio-Products Laboratory, UK
Francesc GODIA	University Autónoma de Barcelona, Spain
Otto-Wilhelm MERTEN	Genethon, France
Steve OH	Bioprocessing Technology Institute, Singapore
Rodney SMITH	CTM Biotech, UK

You will soon receive an election ballot by e-mail. After including your vote, you can send it back the same way, fax or post to me. You will also still be able to vote on site at Harrogate up to the 6th June, i.e. just the day before all ballots will be counted. We hope to receive many votes, thereby showing a high level of participation of all members to the society's life. This election is your opportunity to influence the composition of the Executive Committee and you should not miss it.

The provisional agenda for the General Assembly is as follows:-

Chairman's Report (Otto-Wilhelm Merten)
Treasurer's Report (Martin Fusseneger)
Secretary's Report (Alain Bernard)
Elections of the new committee
Proposal for modification of the constitution
Vote on the new constitution
Proposition and vote for new honorary members
Presentation of the 21st ESACT-Meeting , Dresden, Germany, (Hansjorg Hauser)
Any other business

Every ESACT member is invited to participate in this assembly and we will remind all those attending the Harrogate meeting of this special event

Alain BERNARD, ESACT Secretary

Maintaining Pluripotency of Human Embryonic Stem Cells

Human embryonic stem cells (hESC) hold great potential for regenerative medicine because of their ability to differentiate to any cell type in the body. This potential is retained by keeping the cells in a pluripotent state in long term cultures. Recently, there have been a flurry of papers describing different approaches of maintaining hESC in a pluripotent state which is summarised in Table 1.

In essence, besides the traditional feeder cultures and conditioned media (CM) from feeders; one camp has identified basic fibroblastic growth factor (bFGF) signaling to be important while another group postulate that the transforming growth factor beta (TGF β) signaling pathways are critical. These latest news seems to suggest that hESC are not only pluripotent but perhaps flexible or pliant in their capability to respond to various growth factors in retaining their phenotype. This is a distinctly different paradigm from mouse ES cells which are dependent on leukemia inhibitory factor (LIF) which activates the Jak/Stat pathway. Deciphering the complex pathways and responses these growth factors engender will be an exciting challenge ahead!

Interestingly none of these factors have been identified in CM as yet. However, an attempt has been made by a Proteomics team in Australia to decipher the list of growth factors in CM. (Prowse et al, Proteomics 2005).

Table 1. Growth factors and putative pathways in maintaining hESC pluripotency

Growth factors identified in the support of hESC	**Putative pathways involved in pluripotency**	**Reference**
bFGF alone, hESC seeded at high densities	bFGF signaling?	Draper et al, Stem Cells & Development, 2004
bFGF alone at high concentrations (or with Flt ligand)	bFGF signaling?	CH Xu et al, Stem Cells, 2005
bFGF and noggin	bFGF signaling, suppression of BMP signaling	RH Xu et al, Nature Methods, 2005
TGFβ and bFGF	TGFβ / bFGF signaling?	Amit et al, Biology of Reproduction, 2004
Activin A, nicotinamide and keratinocyte growth factor	TGFβ / activin signaling	Beattie et al, Stem Cells, 2005
Activin A or GSK3β inhibitor	TGFβ / activin signaling, SMAD2/3 activation	James et al, Development, 2005

New €19 Million Institute to Lead Stem Cell Research

Scientists at a newly opened €19m medical centre in Galway have begun research into adult stem cell therapies for a range of serious conditions, including heart disease, spinal cord injury and arthritis. The Regenerative Medicine Institute (REMEDI) at NUI Galway is the leading centre in Ireland doing stem cell research and one of a small number of such centres in Europe combining the technologies of stem cell and gene therapy to regenerate and repair tissue. The researchers believe that stem cell therapy has enormous potential for the treatment of many hitherto incurable diseases, including heart disease and neurological disorders, such as Parkinson's and Alzheimer's Disease.

Early data suggests that the delivery of stem cells to the heart following a heart attack enables regeneration of the damaged tissue and some restoration of function. Dr Frank Barry, Scientific Director of EMEDI, said: 'Adult stem cell treatment is likely to have a dramatic effect on patient recovery and provides us with the potential of treating previously incurable diseases. 'We are very excited about the early data which suggests that the stem cell therapy will be potentially effective in repairing heart tissues.'

REMEDI was established in 2004 through Science Foundation Ireland funding along with industry financing from Medtronic and Charles River Laboratories. The formal opening of the centre was carried out yesterday by Enterprise, Trade and Employment Minister Micheal Martin.

IDA Ireland Chooses UCD to Lead €90 Million Project for Research and Training in Bioprocessing

The IDA has selected UCD to spearhead a €90 Million project to build a National Institute for Bioprocessing Research and Training (NIBRT). IDA Ireland picked the UCD bid, made in conjunction with Trinity College and the Institute of Technology at Sligo, over proposals from Dublin City University and University College Cork.

Ireland's pharmaceutical industry employs more than 17,000 people at 83 bioprocessing operations around the country, accounting for more than €30 billion in exports in 2003. The NIBRT will train bioprocessing technicians, generate research, and serve the needs of institutions and companies engaged in such work. The UCD proposal includes an advanced fermentation facility. The university already has three small bioreactors, along with the Conway Institute of Biomolecular and Biomedical Research, which conducts research into proteins, cell biology, and molecular medicine. IDA Ireland will pick up 90 percent of the estimated €66-million cost of setting up the institute. Operating costs, estimated at €53-million over the first few years, will be met by the university and its partners with help from the private sector and grants from Ireland and the European Union.

Challenges for the Bioprocess Industry (The Third Arms Race)

In a recent article in BioPharm International Dr Stephen Taylor of Avecia predicted that the future of biomanufacturing will be campaigns of ever more complex, personalized biotech-drugs which will drive advances in technology. In exploring this new challenge, he describes two previously described assets: capacity and people.

Capacity

“In recent years most of the focus has been on 'liters of fermentation capacity' — assessment of existing capacity and that under construction with comparison to projected market need. This rather imperfect analysis, based on assumptions about clinical success of products in the global pipeline, generated concerns that lack of capacity would constrain market growth of new products. This view was largely driven by the surge in the numbers of monoclonal antibodies in development and which require very large-scale cell culture biologics capacity. However, recent emphasis in biomanufacturing has moved steadily away from measuring outright investment in stainless steel.

Essentially, this is because it is now recognized that capacity supply and demand have moved into reasonable balance. They seem likely to remain so, with a number of new and expanded manufacturing facilities in North America and Europe being brought online — albeit though there are first signs of potentially un-utilized capacity. These new facilities are the result of investment decisions made in 2001 and 2002, when concerns resulting from Enbrel capacity not keeping pace with demand, with associated loss of sales, first surfaced.

People

Accordingly, increasing attention and media coverage have been devoted to the industry's second challenge — the current and future supply constraints of people and industry-wide competition to secure the brightest and best. The lack of skilled people able to work in biomanufacturing — and, moreover, interested and keen to do so — has been and remains a concern for almost every business either setting up or expanding existing biologics operations. Addressing this major challenge, which is recognizable in so many science-based industries, requires long-term action across the education community and at both national and corporate business levels. In the UK, the plan to establish a national bioprocessing network that will improve interaction between the manufacturing operations of the biotech industry and the academic training and development base is to be applauded and supported.

Through this mechanism the future leaders of our industry will receive inspiration, encouragement and training by exposure to real-life business operating experience. The UK has clearly identified that if it can get the people issues right at a national level and ensure availability of high-quality staff, then business investment and growth of operations should follow. In a similar vein, the recently announced national bioprocessing training institute in Ireland is also a positive step, aimed specifically at the people challenge — and in a country that has received considerable investment in capacity but where the people challenges threaten sustainable growth.

Technology

In one respect, technology is an obvious part of the story. That is, in the context of the growing market and competitive pressure to reduce development timelines and ensure that when new biologics do reach the market, they are economically viable as medicines and profitable to the companies developing them. If we consider some of the conflicting demands on drug development this need is clear. These demands include:

- Global pressure on healthcare budgets is growing with the drugs bill the prime target.
- There is a growing demand in many of the poorer parts of our world for cheaper medicines.
- Many biologics are already at the edge of affordability and are recognized as expensive medicines.
- Without doubt gene discovery will continue to identify many new treatment options.
- Future medicines are almost certainly going to be more complex.....
- .....and we will all continue to demand safer medicines with no side effects.

A focus on increased process and facility productivity is therefore both inevitable and necessary. There are a whole range of technical objectives that are part of the development process. Measures such as antibody titre, process yield, the number of stages of purification and capital investment are but a few of the typical targets for any development program. However, there is perhaps a less well recognized effect of the listed demands above that should influence strategic process research. That is the likely growth of personalized medicines. This is now starting to emerge because:

- Gene discovery will also help identify target populations for specific drugs.

Whilst we should not overestimate the timescale by which this will start to impact — simply because of the very long timescale associated with drug development — it does seem reasonable to assume that our targeting of new drugs to populations where they have an effect will increasingly become a reality. The consequence of such a trend may well be a demand for smaller volumes of more customized biologic medicines — an effect that would erode much of the benefit that large-scale manufacture brings in ultimate production cost.

Thus the pressures on technology not only relate to improved manufacturing productivity and development time, but also to the need to create smarter manufacturing operations able to switch more easily between products and processes — and doing so without compromising on efficacy or patient safety. Speed of response, small-scale manufacturing and process flexibility will become increasingly important. Clearly, this would also place even greater demands and dependency on the skills of the workforce.

Many aspects of our manufacturing processes are crying out for more innovation and

with CMOs growing as the recognized leaders in biomanufacturing, they must drive delivery, working with the regulators to help the industry through a discernable reluctance to adopt new technologies and operating strategies.

It's not hard to see technology as the focus for the next real arms race in biomanufacturing. Meanwhile, we must not forget that the ultimate aim of this strategic contest is to speed up development and contain and reduce the cost of goods because, in the final analysis, making more and better drugs and treatments affordable for, and beneficial to, patients is the real driver for what we do every day."

This article was first published in Contract Services Europe (November 2004, 14-17). Reprinted in Feb 1, 2005.

Future Meetings

2nd World Congress on Regenerative Medicine—From Tissue Engineering to Tissue Regeneration
May 18-29 2005, Congress Center Leipzig, Germany. *www.regmed.org*

Joke Corner

1st April 2005 — It has been officially declared that jokes are banned worldwide due to the lack of humour from a large number of employees who are too busy having their heads to the grindstone, giving cold shoulders to their colleagues and too busy making the world a better place but not a funnier one!

... we must of course take this seriously!

Proposals are now open for research into how to bring humour back into society. Please send them to

sendajoke@humour.org

New ESACT Members

ESACT welcomes the following new members (applications received between October 2004 and February 2005) - see the page opposite. We urge all the new members who haven't done so yet to pay their subscriptions as soon as possible if they wish to register to the ESACT meeting in Harrogate with the member's rate. (log on http://www.esact.org/amember/member.php)

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