

# THE ESACT NEWSLETTER

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## **Editorial - Greetings from the new Team!**

Dear Readers,

Firstly, many thanks to the previous editor Tom Clark for handing over the reins of this readership to the new team.

As the newly appointed Chief Editor of this prestigious society's communiqués, I would like to briefly introduce to you the 3 new incumbents to this newsletter.

The youngest, Lisa Hunt has just completed her Ph.D. at EFPL in Dec. and survived her viva on "The use of fluorescent protein for bioprocess monitoring."

Juergen Lehman hails from the University of Bielefeld. He is 61, been a member of ESACT since 1978. During his academic career he changed his research topic from cultivation of microbes to cultivation of mammalian cells. At the moment his groups are active in 1) Development of robotic pilot plants for mass cell culture for mammalian cells. 2) Production of recombinant proteins including down stream processing. 3) Collaborative research in the field of bioartificial liver support systems. He also teaches Unit Operations of Cell Culture Technology and Downstream Processing, and is the Dean of the Faculty of Technology at the University of Bielefeld.

And after more than 10 years international experience in the life science industry and academia (in animal cell biotechnology, bioseparations, medical devices, clinical trials and medical regulations); I've just returned to the Bioprocessing Technology Centre, at the National University of Singapore to pursue stem cell research, bioprocessing and bioseparations research. I have been an ESACT member since 1989. Below are the smiling faces of Lisa

and Steve...Juergen declined to share his handsome face.

In this issue, we bring to you a variety of scientific perspectives from Switzerland, Germany and Singapore. These include articles on Stem cells, Media development, and a more detailed review of ESACT 17 presentations.

**ENJOY this packed issue!...** And we openly welcome **CONTRIBUTIONS** to the next issue.

**Dr. Steve Oh, Chief Editor**



**Lisa Hunt, Co-editor**



**Joke corner:**

What do you call a lonely and lost gene/plasmid?

*Single stranded DNA, of course!!!*

**A Word from the Chairman:  
O.-W. Merten**

Before all, I would like to express my compassion to our American friends and colleagues, and in particular to those who lost family members or friends during the terrorist attacks on New York and Washington exactly three months ago. I hope sincerely that such terrible incidents can be prevented in the future and that those responsible will be brought to justice, although this will never bring back the victims.

Six months have passed since the 17<sup>th</sup> ESACT Meeting but I expect that all participants still remember the high scientific quality as well as the nice social programme. It will remain in our memories as the Tylösand Meeting organized by Elisabeth. The real organizational end of such a meeting is the delivery of the Proceedings (this is on the way) and the eventual editing of a special issue of a scientific journal (Cytotechnology) with refereed articles selected from high quality oral or poster presentations. The deadline for the submission of the manuscripts has already passed and the reviewing process is on its way.

As soon as one ESACT Meeting is over the organizer of the next Meeting (Francesc Godia from Barcelona/E: the 18<sup>th</sup> ESACT Meeting, Granada/E) finds himself in "la dernière ligne droite" (the last straight line before the aim). The organization of this Meeting is in good hands and on the way to success. During the last ETCS Meeting, which recently took place in Granada, I could visit the city as well as its congress center. The city is very nice, very lively, and there are a lot of

interesting sightseeing possibilities within as well as outside of Granada. The congress center looks very functional, and is quite

professionally organized (as experienced during the ETCS Meeting).

With respect to the meetings following Granada, the ESACT Executive Committee has to plan at least 4 years ahead (up to 2005), the year in which the 19<sup>th</sup> ESACT Meeting will take place. My request in the last Newsletter for propositions for the 2005 Meeting got one positive reply from the UK, meaning that the 19<sup>th</sup> ESACT Meeting will be a British one. Neither the organizing committee nor the site have been chosen yet, however, the future Meeting chairman is already known: Rodney Smith from Xenova in Cambridge. As it has always been the case, the executive committee will support the Meeting chairman wherever possible or necessary.

ESACT is not alone in the world of animal cell technology. As most of you know JAACT, our sister society in Japan, organized her annual meeting in Nagoya City this year. In contrast to previous meetings, this one was called «ASSACT 2001» because it was not only a Japanese but an East Asian Meeting, and for this purpose partially sponsored by the Japanese government. About 80 scientists participated, of which about 10-15 were from abroad (Europe, USA, Iran, Turkey, Pakistan, etc.). The meeting was a scientific seminar without trade exhibition. ESACT was invited to organize a session entitled "Status and future of European animal cell technology". The lectures were the following:

- Overview of 17th ESACT-Meeting (by Elisabeth Lindner-Olsson)
- Metabolic Engineering efforts in Animal Cell Technology (by Francesc Godia)
- Ensuring the viral safety of cell and gene therapy products (by Martin Wisher, BioReliance, Stirling/U.K.)

- Clinical and laboratory surveillance of bovine spongiform encephalopathy (by B. Hornlimann, Public Health Consulting and Research on Prion Diseases in Man and Animals, Zug/CH)

Scientifically spoken, the meeting was good.

A far view to the west takes us to the Americas. With respect to the Engineering Foundation Meetings on Cell Culture Engineering, the number VIII will be organized by M.J. Betenbaugh and J. Aunins in Snowmass/Colorado at the beginning of April 2002 (1-6). To my knowledge the organization is well on the way, the deadlines for oral and poster presentations were the 31<sup>st</sup> of October and the 30<sup>th</sup> of November, respectively. The deadline for registration was the 30<sup>th</sup> of November, 2001.

Since the founding of ACTIP (Animal Cell Technology Industrial Platform) ESACT has been associated with ACTIP and has a permanent observer status in its meetings. This association deals also with mutual information and support with respect to scientific matters. During its last meeting which was held in York/U.K. on the 20<sup>th</sup> and the 21<sup>st</sup> of September 2001, ACTIP decided to revive its task force on Insect Cell Technology. The reasons for this revival are the following:

- Current findings with respect to the risks associated with the Baculovirus/insect cell system for therapeutic/commercial protein production;
- Establishing a group of scientific experts who could analyse the major safety issues and draft recommendations for future investigations where information is lacking or incomplete;
- Develop contacts with national regulatory bodies to understand their concerns and to collaborate in the

preparation of general recommendations for safety testing;

- Prepare a research programme, i.e. within the EC Framework Programmes, to stimulate research aimed at resolving/clarifying some of the safety aspects.

The activities of this task force will be co-ordinated by Helma Hermans (Executive Secretary of ACTIP). In addition, to the interest of at least 12 members of ACTIP in this task force, ACTIP would also like to get more scientific input from ESACT members which are active in the field of insect cell technology. Those ESACT members who are interested to participate in the task force organized by ACTIP should contact me or Dr. Hermans ([actip@wirehub.nl](mailto:actip@wirehub.nl)).

Another point that is potentially of joint interest (ESACT - ACTIP), is the hot topic of training of animal cell technology specialists. All categories (PhD, engineers, and in particular technicians) are in short supply while the industry is expanding as more and more products coming out of the developmental pipeline have to be produced at large scale. Unfortunately I did not get any response to my article in the

last ESACT Newsletter issue concerning this topic, indicating that the question has to be approached more directly. This means that I will contact potentially interested persons in order to get an idea on the true magnitude of the problem. In principle, as a platform of industries active in animal cell technology, ACTIP is also interested in this matter, however, ACTIP has yet to make up its mind on a joint approach with ESACT.

The last call of the 5<sup>th</sup> EC Framework Programme concerning "Cell Factories" (new diagnostics 3.1.1. and therapeutic strategies 3.1.3.) has passed, and every group who has submitted a proposal is waiting very impatiently for a favourable outcome. There

have been issued new calls for TSEs, genomics and newly associated States (the latter with a deadline of the 15<sup>th</sup> of February 2002). Other still open opportunities are calls for fellowships (deadline: 10<sup>th</sup> of April 2002), SMEs (deadline: 16<sup>th</sup> of January , 17<sup>th</sup> of April 2002), accompanying measures (8<sup>th</sup> of February, 12<sup>th</sup> of June 2002) and infrastructures (8<sup>th</sup> of February 2002). For detailed information, see: <http://www.cordis.lu/life/calls/calls.htm>

The 6<sup>th</sup> Framework Programme will definitely be initiated, there is no doubt, although not in the immediate future. The second reading of the proposal will take place in June/July 2002. It is likely that the provisional budget (total budget for the EC will be 16,270 mEUROS, with 13,570 mEUROS intended for research activities, 600 mEUROS for internal co-operation activities, 300 mEUROS for dissemination of results, and 1800 mEUROS for training and mobility of researchers) will be approved.

Thanks to Christophe Losberger and Stephanos Grammatikos the ESACT web site ([www.esact.org](http://www.esact.org)) and JIN are developing to satisfaction. The ESACT website has now a very good presence and it is found in all major directories and search engines. A search with the keywords "animal cell" in any of these search engines usually displays the ESACT web site in the first 20 results. Another good sign is that the welcome page now receives over 1000 hits per week. Thanks to Steph's presentation at the Tylösand Meeting and through direct contacts with the people who had posted vacancies on the hotel board, and also thanks to an overall increase of visitors attracted by the ESACT 2001 web site the number of job vacancies has gone up from 2.2 jobs/month to 6.7 jobs/month. The current vacancies advertized in JIN get presently 74 hits/week. These are very encouraging results and I

would like to thank Christophe and Steph for their effort and to congratulate them for the results.

At the end of this article, I would like to ask our members for input for the nomination of members for ESACT honorary membership. As the name indicates, this membership status should only be offered to people who have performed important achievements in the field of animal cell technology. Your proposition should be accompanied by a short description on the reason why you propose the person for honorary membership.

Finally, I wish you a Happy New Year and for those who live and work in the EURO zone a successful currency change. **Otto-Wilhelm Merten,** Crespières,  
11.12.01

### **A Précis of Stem Cells Knowledge**

Many of you may be wondering what is all the 'hoo-ha' concerning stem cells. In my inaugural article as Chief Editor, I hope to convey a little bit about what is known in this new universe of research, and why it so excites the scientific community. Definitions of key terms and potential research areas will be highlighted.

Scientists interested in human development have been studying animal development for many years. This research yielded our first glimpse at a class of pluripotent stem cells that can develop into almost all of the more than 200 different known cell types in the body. Stem cells with this unique property come from embryos and fetal tissue. In 1998, for the first time, James Thomson at the University of Wisconsin-Madison isolated this class of pluripotent stem cell from early human embryos and grew them in culture. At about the same time new information was emerging about a class of

stem cells that have been in clinical use for years—so-called adult stem cells. Are human adult and embryonic stem cells equivalent in their regenerative potential? Current science indicates that they differ in important ways.

The terminology used to describe stem cells in the lay literature is often simplified or misapplied and even among biomedical researchers, there is a lack of consistency in common terms to describe what stem cells are and how they behave in the research laboratory. To increase the impact of this basic confusion, the field of stem cell biology is advancing at breakneck speed with new discoveries being reported in the scientific literature every week. This

summary begins with common definitions and explanations of key concepts about stem cells. It ends with an assessment of how stem cells research might be applied.

### **DEFINITIONS AND GENERAL CONCEPTS ABOUT STEM CELLS**

A June 2001 report prepared by the National Institutes of Health, USA provides the following key definitions and characteristics of stem cells:-

**A stem cell** is a cell from the embryo, fetus, or adult that has, under certain conditions, the ability to reproduce itself for long periods or, in the case of adult stem cells, throughout the life of the organism. It also can give rise to specialized cells that make up the tissues and organs of the body. Much basic understanding about embryonic stem cells has come from animal research. In the laboratory, this type of stem cell can proliferate indefinitely, a property that is not shared by adult stem cells.

**A single pluripotent stem cell** has the ability to give rise to types of cells that develop from the three germ layers

(mesoderm, endoderm, and ectoderm) from which all the cells of the body arise, see **Figure 1.1 (page 9)**. The only known sources of human pluripotent stem cells are those isolated and cultured from inner cell mass (ICM) of early human embryos and from pre-gonadal fetal tissue.

**An embryonic stem cell** is derived from a group of cells called the inner cell mass (ICM), which is part of the early (4- to 5-day) embryo called the blastocyst. Once removed from the blastocyst, the cells of the ICM can be cultured into embryonic stem cells. These embryonic stem cells are not themselves embryos. In fact, evidence is emerging that these cells do not behave

in the laboratory as they would in the developing embryo—that is, they **CANNOT** develop into a complete living entity or organism.

**An embryonic germ cell** is derived from fetal tissue. Specifically, they are isolated from the primordial germ cells of the gonadal ridge of the 5- to 10-week fetus. Later in development, the gonadal ridge develops into the testes or ovaries and the primordial germ cells give rise to eggs or sperm.

Embryonic stem cells and embryonic germ cells are pluripotent, but they are **NOT** identical in their properties and characteristics.

**Differentiation** is the process by which an unspecialized cell (such as a stem cell) becomes specialized into one of the many cells that make up the body. During differentiation, certain genes become activated and other genes become inactivated in an intricately regulated fashion. As a result, a differentiated cell develops specific structures and performs certain functions. For example, a mature, differentiated nerve cell has thin, fiber-like projections that send and receive the electrochemical signals that permit the nerve cell to communicate with other nerve cells. In the laboratory, a stem cell can be manipulated to become

specialized or partially specialized cell types (e.g., heart muscle, nerve, or pancreatic cells) and this is known as directed differentiation.

**An adult stem cell** is an undifferentiated (unspecialized) cell that occurs in a differentiated (specialized) tissue, renews itself, and becomes specialized to yield all of the specialized cell types of the tissue from which it originated. Adult stem cells are capable of making identical copies of themselves for the lifetime of the organisms. This property is referred to as

“self-renewal.” Adult stem cells usually divide to generate progenitor or precursor cells, which then differentiate or develop into “mature” cell types that have characteristic shapes and specialized functions, e.g., muscle cell contraction or nerve cell signaling. Sources of adult stem cells include bone marrow, blood, the cornea and the retina of the eye, brain, skeletal muscle, dental pulp, liver, skin, the lining of the gastrointestinal tract, and pancreas. The most abundant information about adult human stem cells comes from studies of haematopoietic (blood-forming) stem cells isolated from the bone marrow and blood. These adult stem cells have been extensively studied and applied therapeutically for various diseases. At this point, there is no isolated population of adult stem cells that is capable of forming all the kinds of cells of the body. Adult stem cells are rare (less than 1 in a million cells). Often they are difficult to identify, isolate, and purify. There are insufficient numbers of cells available for transplantation and adult stem cells do not replicate indefinitely in culture.

**Plasticity** is the ability of an adult stem cell from one tissue to generate the specialized cell type(s) of another tissue. Recently, Brazelton et al in *Science*, 2000, reported an example of plasticity where under specific experimental conditions, adult mouse stem cells from bone marrow generated cells that resemble neurons and other cell types that

are commonly found in the brain. The concept of adult stem cell plasticity is new, and the phenomenon is not thoroughly understood. Evidence suggests that, given the right environment, some adult stem cells are capable of being “genetically reprogrammed” to generate specialized cells that are characteristic of different tissues.

**Clonality or clonally derived stem cell.** A cell is said to be clonally derived or to exhibit clonality if it was generated by the division of a single cell and is genetically identical to that cell. In stem cell research, the concept of clonality is important for several reasons. **The importance of showing that one cell type can reproducibly become another and self-replicate cannot be overemphasized.** For researchers to fully understand and harness the ability of stem cells to generate replacement cells and tissues, the exact identity of those cells’ genetic capabilities and functional qualities must be known. Human pluripotent stem cells from embryos and fetal tissue are by their nature clonally derived. However, very few studies have shown clonal properties of the cells that are developed from adult stem cells. It is crucial to know whether a single cell is capable of developing an array of cell types, or whether multiple stem cell types, that when grown together, are capable of forming multiple cell types. For instance, recent research has shown that a mixture of cells removed from fat tissue or umbilical cord blood are capable of developing into blood cells, bone cells, and perhaps others. Researchers have not shown that a single cell is responsible for giving rise to other cell types or, if so, what kind of cell it is. These results may well be attributable to multiple types of precursor cells in the starting tissue; such results from fat cells may, in fact, be due to the presence of haematopoietic stem cells in the fat tissue.

**A progenitor or precursor cell** occurs in fetal or adult tissues and is partially specialized; it divides and gives rise to differentiated cells. Researchers often distinguish precursor/ progenitor cells from

adult stem cells in the following way: when a stem cell divides, one of the two new cells is often a stem cell capable of replicating itself again. In contrast, when a progenitor/precursor cell divides, it can form more progenitor/precursor cells or it can form two specialized cells, neither of which is capable of replicating itself. Progenitor/precursor cells can replace cells that are damaged or dead, thus maintaining the integrity and functions of a tissue such as liver or brain. Progenitor/precursor cells give rise to related types of cells—lymphocytes such as T cells, B cells, and natural killer cells, for example—but in their normal state do not generate a wide variety of cell types.

## **CHALLENGES IN STEM CELL RESEARCH**

It is important to understand some of the difficulties that researchers have had in isolating various types of stem cells, working with the cells in the laboratory, and proving experimentally that the cells are true stem cells. Most of the basic research discoveries on embryonic and adult stem cells come from research using animal models, particularly mice. In 1981, researchers reported methods for growing mouse embryonic stem cells in the laboratory, and it took nearly 20 years before similar achievements could be made with human embryonic stem cells. Much of the knowledge about embryonic stem cells has emerged from two fields of research: applied reproductive biology, i.e., *in vitro* fertilization technologies, and basic research on mouse embryology. There have been many technical challenges that have been overcome in adult stem cell research as well. Some of these barriers include: the rare

occurrence of adult stem cells among other, differentiated cells, difficulties in

isolating and identifying the cells (researchers often use molecular “markers” to identify adult stem cells and grow them in vitro.) Once isolated, one of the major challenges will be to expand and proliferate sufficient of these stem cells in their primitive state for research. Another would be to direct these cells towards functional progenitors for use in clinical relevant applications.

### **WHAT KINDS OF RESEARCH MIGHT BE CONDUCTED WITH STEM CELLS?**

The following are what I would surmise as the major areas of research for stem cells:

1. Transplantation Research— Restoring Vital body functions such as insulin secretion by islet cells, dopamine secretion by neurons, liver function, cardiomyocytes...etc.
2. Therapeutic Delivery Systems for gene therapy
3. Basic Research of embryogenesis and human developmental biology.
4. Functional genomics to investigate specific gene functions in human diploid cells.
5. Pharmaceutical development, to provide large numbers of defined human cell phenotypes for compound screening and toxicology testing.

The new millennium is indeed a launching pad for the combined technologies of genomics, proteomics and stem cell expansion and differentiation, which will auger well for this Animal Cell Technology community!

**Steve Oh, 13<sup>th</sup> Nov. 2001**

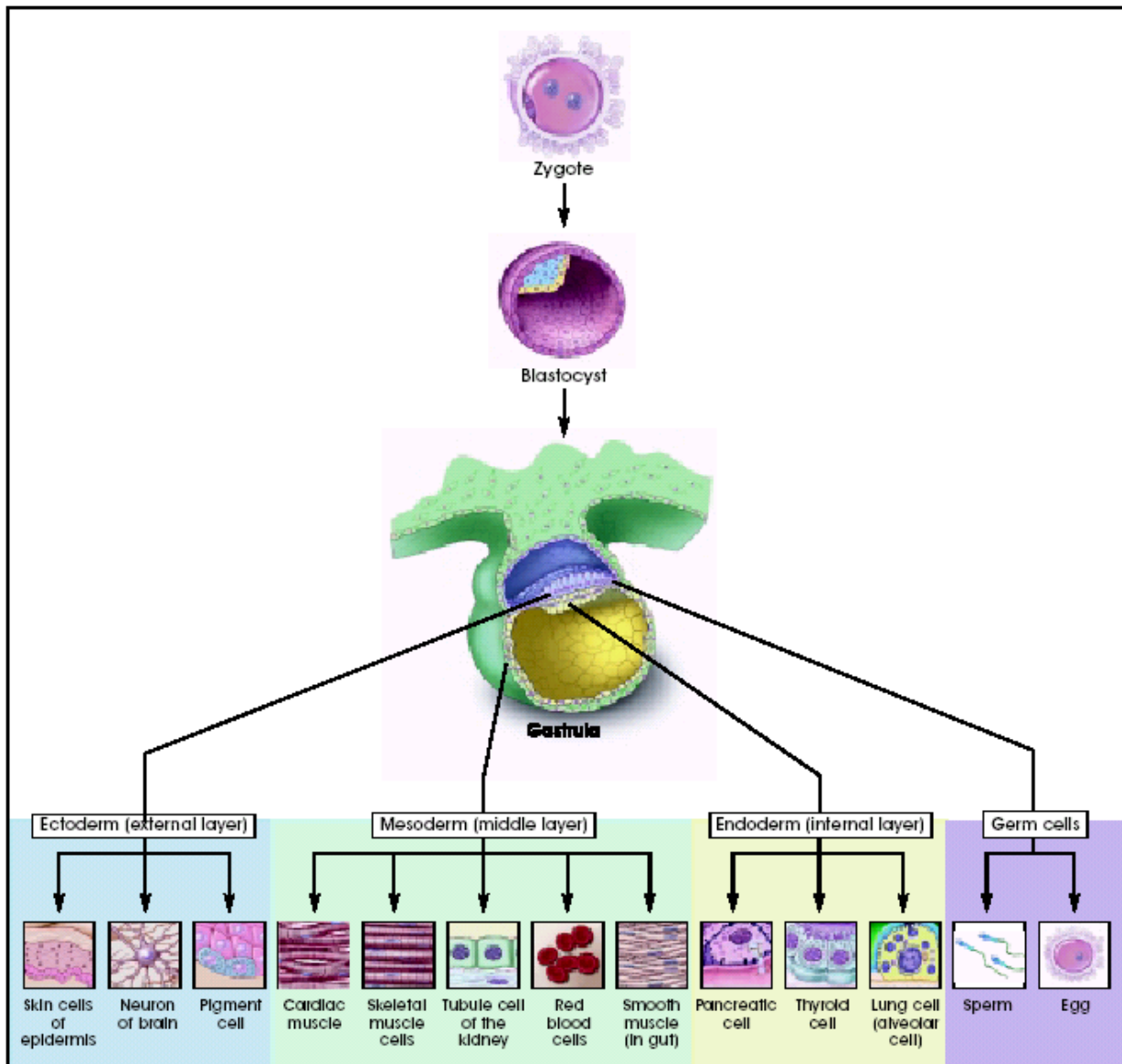


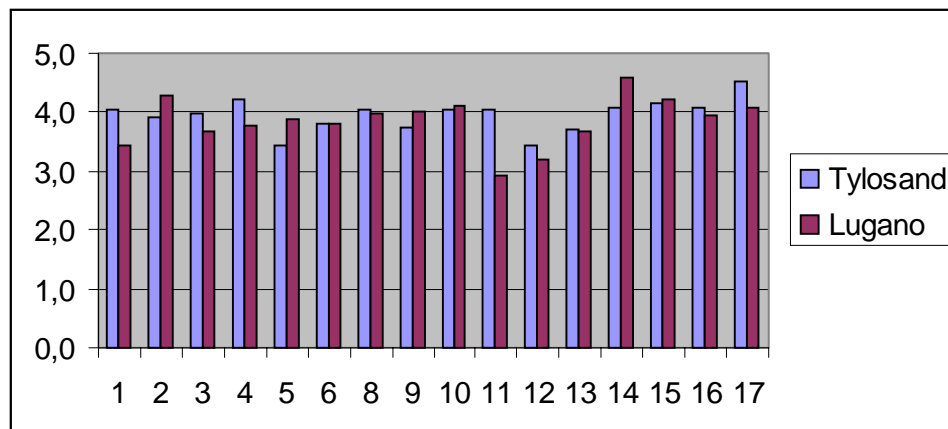
Figure 1.1. Differentiation of Human Tissues.

## Survey Results of Tylösand Meeting

We have now compiled your survey data from the 17<sup>th</sup> ESACT Meeting in Tylösand meeting. Every meeting is graded on a number of parameters; see below, in relation to the previous meeting. This meeting was thus compared with the Lugano meeting. 150 persons out of the 650 participants returned the questionnaire. Both the ESACT Executive Committee and the Organizing Committee for the Tylösand Meeting are very happy to show you these ratings.

In particular we are glad to see the very high scores for **pre-meeting arrangements, keynote lectures, catering** and that the participants are looking forward to our **next meeting** in Granada. However, more **opportunities for young scientists to speak** were requested and the **abstract book** needs to be improved! This encourages us in our ambition to continue to arrange future

ESACT meetings as scientific and social meeting points for people active in the field of Animal Cell Technology.



Average points;

Tylosand 3.95  
Lugano 3.85

1. Pre-meeting arrangements, communication and announcements
2. Location, time of year of the meeting
3. Scientific program
4. Keynote lectures
5. Do you feel that enough "young" scientists had the opportunity to speak?
6. Did you like new sessions such as Identification of Drugs and Drug Targets (1) and Novel Technologies for Administration of Cell Derived Proteins (6)?  
Which new topics would you recommend in the future?
7. Which session did you like the best?
8. Balance of keynotes, orals and posters
9. Size of the audience
10. Organization and assistance throughout the meeting
11. Catering arrangements
12. Registration fee
13. Social program
14. Abstract book
15. Meeting website
16. Overall impression of the meeting
17. Are you considering attending future ESACT meetings?

Elisabeth Lindner-Olsson  
Chair 17<sup>th</sup> ESACT Meeting

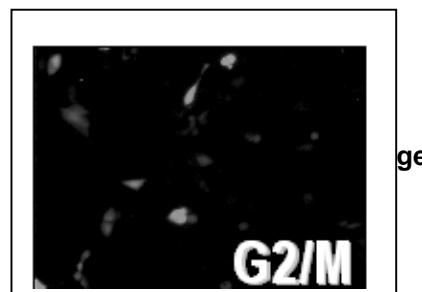
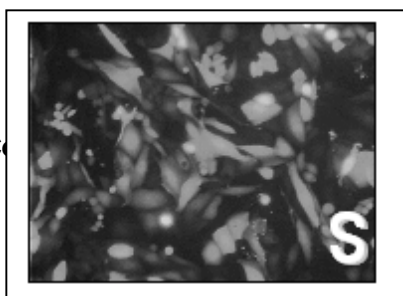
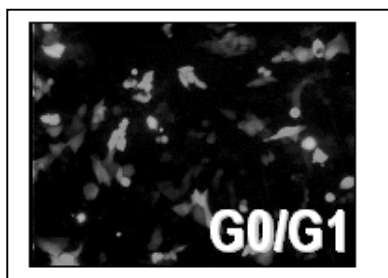
### Winner of 2<sup>nd</sup> prize poster competition at ESACT 17

"S-phase synchronized CHO cells show elevated transfection efficiency and expression using CaPi"

Frederic Grosjean, Laboratory of Cellular Biotechnology, Swiss Federal Institute of Technology-Lausanne, 1015 Lausanne, Switzerland. Email [Frederic.Grosjean@epfl.ch](mailto:Frederic.Grosjean@epfl.ch)  
HTTP: [dcwww.epfl.ch/igc4/](http://dcwww.epfl.ch/igc4/)

### Abstract

When studying transfectability over a 24-hour period after plating, we have observed variable efficiency with calcium-phosphate transfection. This observation on non-synchronous cell population led us to speculate that cell cycle could play an important role in determining susceptibility to transfection by the calcium-phosphate method.



For synchronization of our cell populations, we used mimosine. Mimosine, an iron salt chelator, lowers dNTPs levels available intra-cellularly, preventing at the same time entry of the cell into synthesis phase (Dai et al; Dijkwel et al; Krude; Reichard; Tsai et al.). After mimosine treatment, the majority of cells are arrested at the border between G1 and S-phase. Through optimised addition and removal of mimosine from the culture (a simple wash step with fresh medium), we generated cultures whose cells remain highly synchronized, at least for one, if not two, complete cycles. This synchronized cell populations were transfected at various time points throughout the cell cycle. Through measurement of expression of the fluorescent reporter gene (GFP) we determined the transfection efficiency at these various time points.

Results were presented at the ESACT meeting, showing the effect of the position in the cells cycle upon the transfectability of CHO (DG44) adherent cells. One could see on that poster that, whenever a large percentage of cells were localized in the S-phase, the resulting expression of the reporter gene was much higher than in any other phase. Pictures representing the different parts of the cell cycle, taken under a fluorescent microscope show this effect below. A graph also showed good correlation between reporter gene expression and the percentage of S-phase cells in the synchronized population being transfected. Other mimosine-free synchronization methods were tested, to prevent any artefact due to mimosine. Whatever synchronization method used, the correlation between GFP expression and percentage of cells in S-phase at the time of transfection remained.

## Preparing for the 18th ESACT meeting in Granada: 2003

After the successful meeting in Tylosand, the time has come for the Granada meeting to be organised. The Scientific and Organising Committees are now completed and have started to work on the definition of the meeting sessions, final schedule, keynote speakers, sponsorship, trade exhibition, web site, social programme etc.. Following the trend of the previous meetings electronic submission of abstracts, registration and accommodation will be encouraged through the web site. One change in the schedule is that the meeting will start on the **11th of May at lunchtime**, with a full afternoon session on Sunday, followed by the traders' reception, which will serve as a get together opportunity. The programme will be developed then up to **Wednesday 14th May**, and will end at the Gala dinner. Thus, there will be no session on Thursday morning, and people can plan to travel back from Granada on the 15th. You are deeply encouraged to join the complete meeting, that will have full sessions on Wednesday, just before the Gala dinner when there will be time to enjoy and relax. The next important date will be **May 2002**, when the Second Announcement will be released with more detailed information on the meeting, calling for abstract submission. Be prepared to present your contributions: the final deadline for the abstract submission will be **early December 2002**. Remember that your suggestions are welcomed, especially now that the meeting is in preparation, you can address them directly to the meeting secretary: **francesc.godia@uab.es**. Remember as well to mark your 2003 agendas: the meeting will be in Granada, from 11 to 15 May.  
**Quico Gòdia**

### 17<sup>th</sup> Meeting of ESACT "From Target to Market", Tylösand, Sweden, 10.-14.6.01

An abridged version for the ESACT Newsletter of formal presentations written as an internal report by Hanspeter Amstutz, R&D Molecular Biology, for Zentrallabor Bern.

#### Take home messages

- Apoptosis is the main cause of death in a fermenter culture.
- Gene therapy can be successful, if the target indication/population is chosen appropriately.
- *Ex-vivo* (stem) cell culture and tissue engineering are taking off.

#### Conference talks

In his keynote lecture on stem cells, *L. Philipson* (Karolinska Institute, Stockholm) gave an introductory overview of the current knowledge of stem cells as well as very recent results on the identification of a putative stem cell marker, the receptor for adenovirus which belongs to the Ig super family.

*Topic of Session 1* was the use of large scale, automated screening approaches and was not that relevant for ZLB (Zentrallabor Bern). An interesting twist of phage display peptide library screening was discussed by *B. Buehrer*, who applied the method to discover differences in conformational changes of the estrogen receptor induced by the binding of different ligands (estrogen, Tamoxifen, Reloxin, ICI 192780).

*In Session 2* on new tools/constructs for expression, *A. Morris* (Immunex, Seattle) showed that overexpression of PKB enhanced recombinant protein expression up to 10 fold, through a mechanism that seems related to improved viability. *P. Girard* (EPFL, Lausanne) described a 100 L transient transfection. *K. Lundstrom* (Roche, Basel) reviewed the alphavirus system, which seems suitable for the expression of G protein coupled receptors. *T. Benton* (Corixa, San Francisco) reported

on the use of chromatin opening elements and a cell line preselected for high transfection rate (CHO S) to improve expression. *D. Kompala* demonstrated an inducible expression system based on the glucocorticoid receptor, for which he reported an extraordinarily production rate.

*Session 3* (in memoriam of J. Bailey), started with a comprehensive lecture by *W. Miller* (Northwestern U., Chicago) who comprehensively covered cell metabolism and the influence of various parameters, including pCO<sub>2</sub> and osmolarity, on induction of **apoptosis**. *J. van de Goor* (Genentech) dissected the **apoptotic pathway** in CHO cells. It was shown earlier that 80% of the cells in a bioreactor run die by apoptosis and extension of the lifespan of a culture by only a few days can quadruple the productivity of a production run. In the apoptosis pathway, activation of caspase 3 marks the point of no return. Several inhibitors of caspase 3 were compared to the effective but prohibitively expensive zVAD-fmk.

Over expression of *CrmA* did not show the desired effect, and expression of baculovirus *p35* was problematic, while a mutant of the natural ligand caspase 9 (*caspase 9-DN*) could extend the viability of a bioreactor for 3 days, even when apoptosis was induced by staurosporin.

*M. Al-Rubeai* (U. of Birmingham) presented a different approach to extending a culture: manipulation of the cell cycle. Arrest in G1, achieved by controlled expression of p21, increased productivity 4 fold.

*M. Fussenegger* (ETHZ, Zurich) introduced dual-regulated, inducible expression systems for future applications in gene therapy and tissue engineering.

Metabolic engineering of a plasminogen expressing CHO cell line was performed by the group of *K. Baker*, U. of Kent, by transfection with a regulatable sialyl transferase gene.

Although the physico-chemical analysis showed that they had achieved what they wanted, the biological activity of the product did not increase as expected.

**A highlight of the meeting** was the keynote lecture by *W. Haseltine* (HGS Inc.). HGS has sequenced  $3 \times 10^6$  mRNAs from 1000 cDNA libraries derived from 1000 different human tissues. 90,000 full length mRNAs, originating from 90,000 unique genes, were isolated. This is in clear contradiction to the recently published HUGO and Celera papers, which claim that the human genome has only 30,000 genes. Haseltine: ***“Sequencing the genome is a very difficult way to find genes. The quality of the published gene sequences is not beyond doubt: 75% of the HUGO/Celera genes miss critical exons!”*** HGS has identified 9000 cDNAs of secreted proteins with signal peptides. 8500 of them were unknown before and were not identified by the genome sequencers.

Strategy for further work: 1) Unmet medical need, 2) predictive animal model, 3) high-throughput, automated cell based screening. 6 products identified in this way are in clinical trials.

Keynote lecturer *R. Langer*, (MIT) gave a lively presentation of novel techniques for

tissue engineering and drug delivery (e.g. implantable polymer discs containing drugs for local brain tumor chemotherapy [FDA approved] or implantable chips for electrically inducible drug release [early development]). The latter involved bioerodible polymers to serve as implantable scaffolds for mammalian cells to create new organs or organ parts.

*Session 7* on new therapeutic concepts focused primarily on embryonic or organ derived stem cell therapy, with a promising example of a single adult engrafted stem cell which restored hemopoiesis and gave rise to mesodermally associated cells. Not all details are worked out for routine therapy yet. Three

German laboratories reported on cGMP, *ex-vivo* expansion of autologous cells (DKF Jülich; GBF Braunschweig, MainGen Frankfurt).

Whereas the talk on DNA vaccines against HIV by *D. Weiner* did not induce a lot of enthusiasm, because only marginal success was visible, the lecture of *M. Cavazza*, Paris, on gene therapy of SCID-X1 children demonstrated a success rate of 4 out of 5. The cause of the disease is a genetic deficiency of the  $\gamma_c$  chain common to many cytokine receptors leading to a lack of T and NK cells. An important reason for the success is that T cells with a  $\gamma_c$  chain have a selective advantage and therefore are automatically selected in the patient after  $\gamma_c$  gene transfection of CD34+ cells expanded *ex vivo*.

In the regulatory *Session 4*, John Tong (Lonza Biologics, Slough) talked about Process Range Validation. He demonstrated how a 5000 L process was matrix validated with 18 individual 10 L runs. An important conclusion was that the

specific productivity of the cells was not changed by the variations in process conditions.

*J. Aunins* (Merck, US) shared a proven strategy to improve the efficiency and effectiveness of the transfer of a production process from R&D to manufacturing (avoid unproductive delays and problems in process transfer from R&D to manufacturing: the process must be transferred directly between the parties concerned, and not via an intermediary; R&D needs to be familiar with the production equipment that its process is destined for; Manufacturing has to completely understand the new process and give feed-back on all aspects; Identification of potential problems ahead of time and strategic gathering of data.)

**Hanspeter Amstutz**

## **Special Offer for ESACT-members:**

### **"Recombinant Protein Production with Prokaryotic and Eukaryotic Cells -**

A Comparative View on Host Physiology"

Edited by

**O-W. Merten, D. Mattanovich, C. Lang, G Larsson, P. Neunauer, D. Porro, P. Potsma J. Teixeira de Mattos, J.A. Cole**

The general field of fundamental and applied biotechnology becomes increasingly more important for the production of biologicals for human and veterinary use by using prokaryotic and eukaryotic microorganisms. The papers in the present book are refereed articles compiled from oral and poster presentations from the EFB Meeting on *Recombinant Protein Production with Prokaryotic and Eukaryotic Cells. A Comparative View on*

*Host Physiology*, which was organized in Semmering from 5<sup>th</sup> to 8<sup>th</sup> of October 2000.

A special feature of this meeting was the comparison of different classes of host cells, mainly bacteria, yeasts, filamentous fungi and animal cells, which made obvious that many physiological features of recombinant protein formation, like cell nutrition, stress responses, protein folding and secretion, or genetic stability, follow similar patterns in different expression systems. This comparative aspect is by far the point of most interest because such comparisons are rarely done, and if they are done, the companies who generated them most often keep the results secret.

*Audience:* Presently, a comparable book does not exist because the compiling of manuscripts from all fields of biotechnology (prokaryotic as well as eukaryotic up to animal cell biotechnology) is not done in general. This particularity makes this book very interesting for post graduate students and professionals in the large field of biotechnology who want to get a more global view on the current state of

the expression of recombinant biologicals in different host cell systems, the physiological problems associated with the use of different expression systems, potential approaches to solve such difficulties by metabolic engineering or the use of other host cells, and the co-operation between process development and strain improvement which is crucial for the optimisation of both the production strain and the process.

**November 2001, Hardbound, 410 pp., ISBN 0-7923-7137-2**

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## **Web Discussion Board**

A new feature has recently been installed at [www.esact.org/board.html](http://www.esact.org/board.html), the ESACT Discussion Board. This forum is open to all to discuss any subject related to cell technology and science in general.

We hope that many researchers will participate in these open discussions and that it will become a place of reference to ask technical and regulatory questions, compare technologies, or share views on ethical or political problems. This is the perfect place to carry on the fruitful discussions that start at the ESACT meetings!

The ESACT discussion board was implemented for the use of the community and can only be useful with the help of the community, so please promote it in your labs, institutes, departments and companies!

Address any question about this new feature to [webster@esact.org](mailto:webster@esact.org)

## Gene Vectors Euro Lab Course

An invitation to the reader to the Gene Vectors Euro Lab Course: *This is the first multidisciplinary theoretical and practical training in vectorology. Généthon will organize this practical and theoretical training course in the frame of VecEuroNet in Evry between the 14<sup>th</sup> and the 27<sup>th</sup> of April 2002. This course is sponsored by the European Commission, EMBO, INSERM, and the Journal of Gene medicine:*

Successful clinical gene therapy imposes the need for fundamental understanding of vector biology and production parameters such as purification, quality control, optimisation of titres, and regulatory issues. By combining the above, scientific and technological innovations can be transformed into commercial success.

Our inaugural multidisciplinary *Euro Lab Course* will have high-level theoretical and practical training in vector production, lectures covering ethical and legal issues related to gene transfer, and how bench-manufactured vectors can be transformed into medicine by industries. Seminars

on major viral and synthetic vector and a biotech forum will gather international scientists from academia, industry and regulatory agencies. Furthermore, hands-on training on synthetic and viral vectors will train 30 young European researchers selected on the basis of scientific excellence.

**PLEASE SEE ENCLOSED LEAFLET FOR FULL DETAILS OF THE COURSE WITH PROGRAMME AND DEADLINES**

## UPCOMING MEETINGS

### **THE 18<sup>th</sup> ESACT MEETING**

**May 2003 – GRANADA SPAIN**

**Complete information from;**

[www.esact.org/esact2003/](http://www.esact.org/esact2003/) or  
[gp@pacifico-meetings.com](mailto:gp@pacifico-meetings.com)

### **JAAC T 2002**

**Nov 11 – 15, 2002**

Fuchu City, Japan

[www.tuat.ac.jp/~jaact02/](http://www.tuat.ac.jp/~jaact02/)

## NEW MEMBERS

We would like to welcome to ESACT the following new members;

Sanjay Adagoor (IWST, India), Richard Allread (Serologicals Ltd, UK), Torben Bachmann (Novo Nordisk, Denmark), Augustine Bader (GBF, Germany), Jingxiu Bi (GBF, Germany), Lorraine Buckberry (Scancell Ltd, UK), John Crowley (DSM, Holland), Samuel Denby (UCL, UK), Lisa Hunt (EPFL, Switzerland), Nazlee Kamal (Sartorius, Malaysia), John Kastrup (Bie & Bernstein A/S, Denmark), Mohammed Mohammed (Novartis Animal Vaccines, UK), Heikki Ojamo (MediCel Oy, Finland), Ralph Otto (Boehringer Ingelheim, Germany), Patrick Ziltener (Actelion Pharmaceuticals Ltd, Switzerland).