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Meeting report

Recombinant Protein Production: A Comparative view on host physiology (Laupheim, Germany, March 2013)

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The '7th Conference on Recombinant Protein Production – A Comparative view on host physiology' was held in Laupheim (Germany) in March 2013. It focused on different aspects of analysis, engineering and control of cellular physiology for recombinant protein production with a focus on proteins that are difficult to produce. Today recombinant protein production is a standard technology in all areas of biotechnology as well as in basic life sciences. The quantitative yield and the quality of the product are strongly dependent on the physiological status and competence of the biological catalyst, which is a complex cellular network in most circumstances. Thus, although brute-force strategies are commonly applied and have been successful in many cases for a large number of target proteins, it is more and more recognized that Quality by Design (QbD) approaches that include the molecular design of the cell and the distinct regulation of its components (i.e. the quantitative balance of all components in the network) are the key for high reliability and reproducibility of bioprocesses. This is of utmost importance for industrial scale up, where the environment in a reactor changes not only over the process time but also in space. A deeper understanding of the cellular system is especially important if cells are engineered to perform co- and post-translational modifications of target product and if the target protein is composed of different subunits or even large protein complexes.

This strong interconnection between protein production and cellular physiology of the production system, and the integration of both prokaryotic and eukaryotic systems, is a unique feature of the series of the 'Recombinant Protein Production: A Comparative view on host physiology' biennial events organized by the Microbial Physiology section of the European Federation of Biotechnology. The 7th conference in this series, which for the first time was hosted by a Biotechnology company, Rentschler Biotechnologie GmbH, attracted almost 200 participants from academia and industry from 25 different countries including participants from the USA, Japan, South Africa and Israel. The participation of over 60 participants from industry from more than 45 companies, the gratefully acknowledged sponsorships from Rentschler Biotechnologie, Novo Nordisk, DASGIP, Stanstead Fluidic Power, Sanofi and VTU Technology, cash prizes for the best posters by young scientists sponsored by Novozymes, and participation of exhibitors with novel technologies (IBA Life Sciences, Wyatt Technology Europe, SynenTec Bio Services, Optocell, BioSilta, PreSens), nicely illustrate the idea of this event, to bridge and channel the progress in academic research to the industrial production of recombinant proteins.

Including the preconference session on Biomolecular Technology of Proteins, the 43 oral presentations included three plenary lectures by Alfred Pühler (Center for Biotechnology, Bielefeld University, Germany), Lloyd Ruddock (University of Oulu, Finland) and Louise Glass (The University of California, Berkeley, USA) and eight invited lectures that spanned process and product quality control, strain to process design, production of difficult proteins, analysis and control of cell metabolism, engineering gene expression and regulation, and strategies to enhance protein folding, transport and secretion.

A Comparative view on host physiology

The production of recombinant proteins is a key technology in most life-science projects, independent on the application and the target. Often it is important to produce a desired protein in large quantities, like biocatalysts for industrial, environmental or pharmaceutical applications, demanding a cheap end-product and an efficient process. Other projects rely on an ultra-pure grade of the protein of interest, as for instance in structural studies as a basis for drug design or understanding the function of proteins in their biological environment. In all these cases it is important to have a well characterized expression host, a well documented and readily available expression system and an efficient procedure for screening to identify the best conditions for production.

Bacteria are the favorite host for the production of structurally simple proteins that are easy to produce. Mammalian proteins often require complex post-translational modifications

1871-6784/$ - see front matter http://dx.doi.org/10.1016/j.nbt.2013.06.002

like N-glycosylation or complex disulfide bond pattern which are so far not commonly realized in bacterial systems and generally demand eukaryotic systems like CHO-cells or yeasts. However, new exciting advances in bacterial glycosylation, control of disulfide bond formation and in the production of protein complexes have been made recently and are starting to be applied successfully for the production of more complex proteins. These new systems, strategies and experiences pave the way to produce proteins in bacteria that have previously been considered to be difficult or even impossible to produce in prokaryotic systems. Especially for such systems with newly engineered pathways, but not limited to them, the detailed understanding of the physiology of the cells is a key to guarantee a high production yield and quality of the target protein. This requires not only its accumulation as a correctly folded protein and with the correct modifications, but also accumulation as a homogeneous fraction with a low structural variability. Quality control can be obtained by tuning the rate of protein synthesis with the expression of the co- and post-translational modification systems and the folding rate of the target protein. Most importantly, these parameters are closely bound to the metabolic state of the cells, which is defined by the culture conditions. In this sense RPP7 aimed to discuss recent breakthroughs in engineering, control and understanding of growth physiology in both established microbial and eukaryotic hosts as well as in new expression hosts. The meeting also discussed specific targeting to different compartments and controlling post-translational modifications. The target protein groups represented the challenging proteins today: large protein complexes, membrane proteins and antibodies.

Understanding growth – new insights on host physiology

The detailed understanding of the growth behavior of a host cell is crucial to maximize the yields in a recombinant protein production process in qualitative and quantitative terms. Especially interesting are physiological responses toward stress situations such as environmental stresses or to the induction of recombinant protein production, as these may be connected to bottlenecks in the cellular production network.

The importance of understanding the physiological system of an expression host was clearly documented by Roslyn Bill (Aston University, Birmingham, UK) using as examples human membrane proteins that are normally obtained only in a low yield when expressed in the yeast *Saccharomyces cerevisiae*. Her group successfully removed physiological bottlenecks in heterologous protein expression via transcriptomic analyses and engineering approaches, and at the same time revealed new insight into translational and post-translational processes. Their powerful approach included the strategic use of experimental design (DoE) instead of the common strategy of changing one factor at time (OFAT). Bill and her group identified by their systematic approach several genes that are connected to high yielding strains, usually genes upregulated at low cultivation temperatures. Overexpression of one of these genes, *BMS1*, resulted in higher functional yields for membrane proteins. Furthermore, the authors could improve the production of membrane proteins significantly under standard cultivation conditions by the use of a fully respiratory mutant strain of *S. cerevisiae* without the need of complex control of cultivation conditions. This is a significant improvement compared to the standard, where improved yields could only be obtained at low cultivation temperatures.

Interestingly, filamentous fungi show also an increased level of recombinant protein production at low growth rates. *Trichoderma reesei* is the most widely used expression host for industrial enzymes applied in the biofuel, food, textile, and paper production sectors. Markku Saloheimo (VTT, Finland) illustrated the effect of the growth rate on the protein production physiology. Major biosynthetic activities at higher growth rates have a negative impact on protein expression, whereas secreted protein expression and secondary metabolism, which are upregulated at low growth rates, have a positive correlation.

A further focal point was the commonly used yeast *Pichia pastoris*. *P. pastoris* is a well-established organism for research and industrial applications. It is useful because of its high potential to secrete recombinant proteins whereas endogenous proteins in the cultivation medium are only found at very low levels. A drawback of *P. pastoris* in many cases is the low yield of the recombinant protein in the cultivation medium. Several talks presented the current advances in finding essential clues for more efficient protein expression in *P. pastoris*. The group of Diethardt Mattanovich (BOKU, Vienna, Austria) introduced their work on a metabolic model of *P. pastoris* based on the completed genome sequence and the incorporation of heterologous protein production. This new approach enabled the interactions between protein production, energy demand and biomass formation to be investigated. The group of Pau Ferrer from the University Autònoma de Barcelona (Spain) verified via ^{13}C-Metabolic Flux Analysis increased glycolytic, TCA-cycle and NADH regeneration fluxes in a recombinant *Pichia* strain compared to a control lab strain.

Besides understanding growth, fast generation of reliable and efficient expression strains is important for industrial approaches. Roland Weis (VTU Technology GmbH, Austria) presented a versatile promoter library for target and auxiliary genes that can give rise up to 25,000 individual *P. pastoris* mutant strains and high-throughput methodologies to screen such a library within one week.

In addition to yeast *Escherichia coli* is a favored expression host, because it combines the capability to reach high-cell densities with simplicity in cultivation. A downside of *E. coli* is the formation and accumulation of acetate by overflow metabolism, which has a negative impact on cell growth and protein production. Zhaopeng Li from the group of Ursula Rinas (Leibniz University of Hannover, Germany) could show that an *E. coli* strain not forced to produce recombinant protein is able to re-assimilate all produced acetate when entering the stationary growth phase due to upregulation of coenzyme A synthase and TCA-cycle enzymes. In protein-producing *E. coli* strains these enzymes are expressed at a lower level compared to non-producing strains. Jürgen Mairhofer (BOKU, Vienna, Austria) presented a very interesting new result on the story why *E. coli* B-strains show lower acetate production under excess nutrient conditions compared to *E. coli* K-12 strains. *E. coli* BL21 has a different expression pattern of membrane porins due to the absence of the *ompC* and *mglB* (inner membrane) genes. An *E. coli* K-12 knockout double mutant for *ompC* and *mglB* reveals similarly low acetate production levels as *E. coli* B-strains.

How very small changes in the type of an expressed protein may affect the kinetics of an *E. coli* culture was presented by Natalie Rahmen from the group of Jochen Büchs (RWTH Aachen, Germany). As shown with on-line measurement of the oxygen transfer rate in a RAMOS device, the authors demonstrated that single amino acid exchanges in the sequence of the recombinant protein mediate significant differences in respiration activity and recombinant protein production, yet such approaches have still to be correlated with the folding characteristics of the mutants.

A major current bottleneck in the development of new bioprocesses is the use of different cultivation techniques in the product screening phase and in process development. Whereas the final processes are always fed-batch fermentations, the initial screening is mostly performed in batch systems. Novel solutions such as EnBase® (BioSilta, Oulu, Finland), FeedBeads (Kuhner, Birsfelden, Switzerland) and Feed-In-Time (M2P-labs, Baesweiler, Germany) media provide the opportunity for a consistent use of the fed-batch technology through all developmental stages and provide the additional benefit of high cell densities. Csilla Török (ACIB GmbH, Austria) demonstrated the comparability between such fed-batch-simulations in microwell plates and fed-batch fermentations in lab-scale bioreactors. In addition a single-use rocking system developed by CELLution Biotech (Netherlands) was presented by the CEO of the company, Nico Oosterhuis. The CELL-tainer®, which is available now in two versions for 15 L and 150 L cultivations, has a superior oxygen transfer rate (k_La of 200–400 hour^{-1}) and therefore can be used for bacterial high cell density cultivations. In cooperation with the group of Peter Neubauer (TU Berlin, Germany) *E. coli* final biomass concentrations up to 45 g L^{-1} and good production yields of a recombinant product were obtained.

Michael Jahn (Helmholtz Center for Environmental Research Leipzig, Germany) studied the development of subpopulations in cultures of *Pseudomonas putida* expressing a recombinant EGFP protein using a novel combination of flow cytometric cell sorting followed by mass spectrometry based proteomic analysis. There were only minor differences in metabolism between low- and high-producing subpopulations, but several proteins connected to stress responses were highly upregulated in the EGFP expressing subpopulations.

In the closing lecture, Louise Glass (University of California Berkeley, USA) presented fascinating data on transcriptomic and proteomic studies with the filamentous fungus *Neurospora crassa*. *N. crassa* is an interesting organism due to the ability to degrade plant material. Glass and her colleagues have studied the secreted proteins (secretome) and their expression patterns during the growth on cellulose, pectin and hemicellulose as well as during the growth on intact plant cell walls. Manipulation of transcription factors regulating these enzymes enabled inducer-free production and secretion of these enzymes. Furthermore, transferring degrading enzymes and transporters from *N. crassa* into *S. cerevisiae* resulted in yeast strains able to transform cellodextrins into ethanol for improved biofuel production.

If the desired recombinant protein needs complex post-translational modifications higher eukaryotic expression systems based on insect or mammalian cells are the preferred choice as production cell factories. Also to increase the yield of produced protein using these cell lines, it is important to understand the physiological effects of recombinant protein production on the host cell. A milestone is the availability of the CHO (Chinese Hamster ovary) cell genome. Alfred Pühler (Bielefeld University, Germany) introduced state-of-the-art high throughput and next-generation sequencing approaches for genomes and transcriptomes of CHO cell-lines to provide new information on the activity of genes and how they are transcribed. In addition the group of Johannes Grillari (BOKU, Vienna, Austria) characterized the miRNome of CHO cells. Micro RNAs influence cell behavior globally as one specific miRNA-species can regulate the translation of up to 100 mRNAs. What makes them special is the fact that miRNAs are not translated and therefore are no additional translational load for protein production. Results of the study have shown that miRNAs can be a tool for cell engineering and are able to improve biotechnologically relevant CHO-cell characteristics. The importance of systems biotechnology approaches to understand cell physiology for predictive process improvement was presented by the group of Paula Alves (Universidade Nova de Lisboa, Oeiras, Portugal). CHO cell clones expressing variable amounts of a monoclonal antibody were cultured under several conditions. ^{1}H NMR analysis of supernatant compounds during the cultivation allowed the estimation of metabolic fluxes and the creation of a flux network of 117 reactions. Based on the analysis results supplementation strategies for the culture have been designed and validated, yielding to a higher specific productivity.

Broadening the horizon – improved known and new expression systems

Although these new insights on CHO-cell physiology may help to improve bioprocesses, a current drawback is the random integration of the genes of interest. This requires time-consuming screening for cells to discover where this integration happened in a stable and highly active genomic locus. Claas Wodarczyk (Rentschler Biotechnologie, Laupheim, Germany) presented a novel cell line development platform in which the gene of interest is integrated into pre-characterized gene loci. Thus cell lines with predictable behavior and protein production can be produced for any target protein.

As described before, filamentous fungi are high potential cell factories for recombinant proteins and secondary metabolites. Vera Meyer (TU Berlin, Germany) introduced her results on trying to decouple recombinant protein production and biomass accumulation. Here group established a retentostat cultivation protocol for *Aspergillus niger* providing stable nutrient supply to a high cell density culture of *A. niger* at without growth and characterized two new promoter candidates for recombinant protein production that are highly upregulated at such conditions.

Novel regulated promoters for high-level expression in *P. pastoris* were presented by a group from the BOKU (Vienna, Austria). For safety reasons the use of methanol in industrial scale processes is a major disadvantage. Hence there is demand for novel methanol-free inducible promoters for industrial applications of *P. pastoris*. Via DNA microarray analysis six promoter candidates with desired characteristics were found with the two strongest promoters achieving an exceeded productivity compared to the established methanol-free P_{GAP} promoter.

Besides improving established host organisms, several new organisms were introduced as hosts for efficient recombinant protein production. Casper Jørgensen (Bioneer A/S, Denmark) described an expression system based on the Gram-positive endotoxin free bacterium *Lactococcus lactis*. The system is lactose-inducible and the organism is able to secrete heterologous proteins into the cultivation medium. This bypasses the problem of inclusion body formation in the cytoplasm, which is very common, for example, in *E. coli*. Another possible candidate for future large-scale protein production is a *sipY* mutant of *Streptomyces lividans*. A group from UAB (Barcelona, Spain) discovered that although *sipY* plays a major role in preprotein processing in the secretion pathway, such a mutant strain also has low extracellular protease activity and for this reason is a potential host for overproducing biotechnologically interesting proteins.

A newly and metabolically engineered *Bacillus subtilis* strain based on *B. subtilis* ATCC 6051 was presented by Thomas Schweder (Ernst-Moritz-Arndt-University Greifswald, Germany). *B. subtilis* ATCC 6051 shows more desired growth properties compared to common lab strains. To turn this strain into an alternative expression

host its genome was sequenced, and lysis-reduced protease-deficient non-sporulating mutants were created. As *B. subtilis* is not able to utilize its own overflow metabolites acetate and acetoin, the glyoxylate shunt from *Bacillus licheniformis* was transferred leading to a *B. subtilis* strain displaying improvements in growth and protein expression in acetoin induced cultivations.

Another utilized strain from the *Bacillus* genus was introduced by Michael Crampton from the CSIR (Pretoria, South Africa). The halo-, thermo- and basophilic bacterium *Bacillus halodurans* naturally produces flagellin at high levels. To use these flagella for the surface display of recombinant peptides and proteins the natural flagellin coding *hag* gene was inactivated and replaced by a hag variant with inserted peptides or proteins. This engineered mutant named *B. halodurans* BhFC07 was shown to be able to display a poly-histidine peptide, HIV epitope, cellulose binding protein and metal binding proteins on its cell surface.

Supporting downstream processes – new insights on secretion of recombinant proteins

Downstream procedures to purify produced recombinant proteins can consume up to 80% of the total process costs. When the desired protein is produced in the cytoplasm the disruption of the cell is the first step in downstream processing. Afterwards the recombinant protein has to be purified from the crude cell extract including host proteins, lipids, nucleotides, sugars and other cell components. To bypass these cost-intensive treatments efficient secretion hosts can be engineered, which allow the separation of the desired protein from the biomass via simple filtration or centrifugation steps. Advances in the area of prokaryotic and eukaryotic protein secretion systems were also presented at the conference.

In addition to the surface-display strain *B. halodurans* BhFC07 Michael Crampton (CSIR, Pretoria, South Africa) introduced a further mutant *B. halodurans* BhFDL05S. This mutant is sporulation and lysis deficient and numerous proteases have been knocked-out. Furthermore, the flagellin polymerization protein was inactivated turning the flagella production into an efficient recombinant peptide secretion machinery. The group is now looking for suitable expression promoters for efficient high-yield protein production.

Yeasts are also favored hosts for developing secretion systems for protein production, as they have well characterized secretion pathways. A great effort has been put on understanding and engineering these pathways to give rise to enhanced capacities for folding and trafficking of proteins and improved secretion. But the secretion yield of recombinant proteins depends on their physicochemical properties. In a study presented by Novo Nordisk (Denmark) two model proteins, which only differ in minor amino acid changes, were produced in *S. cerevisiae*. Although no difference in the production of the proteins could be found when they were expressed in the cytosol, a sevenfold difference in secretion yield was measured. Physiological characterization of cells expressing both model proteins revealed differences in the overflow metabolism and protein maturation. Hence the changes in the conformational stability of the recombinant proteins due to the amino acid changes lead to large differences in secretion yields. In addition a group from the Saarland University (Saarbrücken, Germany) revealed via proteome analysis of *Schizosaccharomyces pombe* an unexpectedly high amount of changes in the regulation of the central carbon metabolism and amino acid synthesis during protein production. Finally secretion could be improved by feeding of the amino acids that are needed in a higher ratio in a recombinant protein compared to the general host proteins.

Although *E. coli* is generally not considered as a primary host for the secretion of heterologous proteins, in the past some secretion systems have been described for this organism. Here, Joen Luirink (VU University Amsterdam, Netherlands) introduced autotransporters, which are virulence factor proteins secreted by Gram-negative bacteria. It is suggested that these proteins carry all information needed for the translocation from the cytoplasm to the outside of the cell. He illustrated molecular details of the three protein domains that enable the translocation process through the inner membrane, the periplasm and the outer membrane and attempts to use this system for the secretion of heterologous peptides.

Amanda Rossiter (University of Birmingham, UK) presented a novel *E. coli* based autotransporter secretion system combined with a high throughput screening approach to generate *E. coli* strains that are stress-resistant and secreting the desired protein in high-yields. The industrial potential of *E. coli* based secretion systems was well documented with the example of the ESETEC® system, which is commercialized by Wacker Biotech (Jena, Germany). Using this system it is possible to produce secreted recombinant proteins with yields comparable to common inclusion body-based processes, but with an improved recovery. In addition to the ESETEC®-strains a toolbox was developed to enable the production of difficult proteins. A more individual approach in finding secreting *E. coli* strains was illustrated by Karl Friehs (Bielefeld University, Germany). His group induces actively mutations in the recombinant strains via UV-light or chemicals followed by a screening for enhanced secretion of the desired protein. Protein secretion was supported by coexpression of bacteriocin release proteins, which influence the composition of the outer membrane and ease the secretion of proteins from the periplasm into the medium.

Advanced recombinant proteins – studies on difficult-to-express proteins and protein-complexes

Besides the view on host physiology the RPP7 conference focused on the issue of multiprotein complexes, large multifunctional proteins, membrane proteins and disulfide bond containing proteins. Various innovative approaches were applied to express such challenging proteins with high solubility and functionality.

To understand the structure and function of eukaryotic multiprotein complexes, it is necessary to produce them in sufficient quality and quantity for purification, crystallization and molecular studies. However, the conventional cloning strategies are not qualified to produce larger protein complexes, usually with several hundred kDa. To circumvent this bottleneck, Imre Berger (EMBL Grenoble, France) as an invited lecturer presented a novel and powerful baculovirus expression vector system (MultiBac), that is, particularly tailored for the production of eukaryotic multiprotein complexes. With the MultiBac system many important proteins and their complexes, for example, human general transcription factor TFIID core complex, a tumor suppressor kinase LKB1–STRAD–MO25 complex, and a DNA damage checkpoint Rad9–Rad1–Hus1 complex have already been successfully expressed and analyzed. Melanie Kern (TU Darmstadt, Germany) reported recombinant production of a bacterial quinone/quinol-reactive metalloprotein complex in *E. coli*, which is essential to catalyze global nutrient (e.g. nitrogen and sulfur) cycles. The difficulty for producing this quinone/quinol-reactive protein is that a co-factor like Fe/S cluster has to be co-expressed and incorporated into the protein. As an example the NapGH quinol dehydrogenase complex was actively produced with coexpression of a Fe/S cluster *iscSUA-hcsBA-fdx*. Timm Fiebig (Hannover Medical School,

Germany) discussed expression and characterization of the capsule polymerase of *Neisseria meningitidis* serogroup X. The *xcbA* gene (serogroup X capsule biosynthesis) was expressed in *E. coli* and the resulting protein was able to produce polysaccharide capsules in milligram quantities. In addition, a talk on the successful expression in *P. pastoris* of four *N. crassa* lytic polysaccharide monooxygenases was given by Daniel Kracher (BOKU University, Austria). The produced enzymes could be possible for biofuel production from lignocellulosic biomass in the future. Jian Li (TU Berlin, Germany) illustrated how to make very large proteins and keep them active in *E. coli*. The multifunctional mega-enzyme nonribosomal peptide valinomycin synthetase (655 kDa) was actively expressed for the antibiotic valinomycin formation. The production of valinomycin could reach milligram per liter levels using the fed-batch type EnBase® cultivation technology.

Important drug targets – membrane proteins

Membrane proteins play significant roles in all essential cellular processes such as the uptake of nutrients, the export of toxins and the cell–environment interactions. It is believed that around 50% of pharmaceutical drugs target membrane proteins for a broad range of human diseases. Therefore, gaining a structural and functional understanding of membrane proteins is pivotal to the future of new drug design and discovery. However, membrane proteins are not naturally abundant in their native membranes making direct purification and further biochemical studies difficult. Therefore it is a very important question of how to make membrane proteins in sufficient quantity and of appropriate quality for further studies. In addition to the already mentioned studies by Roslyn Bill, Fabrizia Fusetti (University of Groningen, The Netherlands) presented a directed evolution method for optimized production of recombinant membrane proteins. This method involved fusion of GFP in tandem with the erythromycin-resistance protein ErmC at the C-terminal of the target protein. The GFP fluorescence allowed screening for correct folding, and thus functionality *in vivo*, whereas ErmC is used to select for increased expression. Higher expression levels were achieved by gradually increasing the erythromycin antibiotic concentration in the medium.

In the invited lecture, Frank Bernhard (Goethe University Frankfurt, Germany) provided another view on membrane proteins production with cell-free systems. He gave an overview on the preparative scale cell-free production of G-protein coupled receptors, transporters and membrane integrated enzymes involved in peptidoglycan synthesis. Co- and post-translational modulation of the protein environment was systematically used in order to optimize membrane protein activity and stability. The enzymatic activity of membrane proteins could be strongly modulated by the type of lipids provided with the nanodiscs.

Tight connections – advances in recombinant antibody production

Veronika Chromikova (BOKU, Vienna, Austria) compared production of recombinant monoclonal IgM in two different mammalian CHO-DG44 cell lines: M617 and M12. The specific productivity in the M12 cell line was significantly lower than in the M617 cell line, despite using very similar plasmid constructs. To explain the difference, relative gene copy number and transcription levels were measured from the two cell lines. The results indicated that the M617 cell line had a higher relative gene copy number, whereas M12 manifested a higher relative transcription efficiency. As this does not explain the specific productivity results, a hypothesis was proposed that M12 encounters difficulties during protein folding inside the endoplasmic reticulum. Thus the antibody stays inside and causes increased cellular stress. Karin Felderer (MorphoSys, Germany) presented the new technologies for generation of recombinant antibodies in bacteria based on the Human Combinatorial Antibody Library (HuCAL). This technology also enables *in vitro* selection and functional characterization of antibodies with a versatile antigen vector toolbox.

Understanding and implementing post-translational modifications

One of the most important post-translational modifications found in proteins is disulfide bond formation, which plays an essential role in the folding and stability of some proteins. Because the formation of native disulfide bonds is often the rate-limiting step of protein biogenesis, it is still a challenge to produce disulfide bond containing proteins on a large scale. Lloyd Ruddock (University of Oulu, Finland), the meeting opening plenary lecture speaker, gave a comprehensive report on mechanisms and applications of disulfide bond formation in the cytoplasm of *E. coli*. In his group a promising system called CyDisCo (cytoplasmic disulfide bond formation in *E. coli*) was developed, allowing efficient disulfide bond formation through combining the pre-expression of a sulfhydryl oxidase (Erv1p) and a disulfide isomerase in the cytoplasm of *E. coli*. Current versions of the CyDisCo system, while still not optimal for all proteins, can easily be transferred between bacterial strains and allow the production of human proteins with multiple disulfide bonds in *E. coli* grown in shake flasks with yields of up to 100 mg l^{-1} culture. Alexander Daniel Frey (Aalto University, Finland) described a novel synthetic N-glycosylation pathway to produce recombinant proteins carrying human-type N-glycans in *S. cerevisiae*. Using the double mutant strain *Δalg3Δalg11*, a protein bound Man3GlcNAc2 can be produced and serves directly as a substrate for Golgi apparatus targeted human N-acetylglucosaminyltransferases I and II creating human-type N-glycan structures.

Future plans

The next conference in this series (RPP8) will be held in March 2015 near the EFB Central Office in Barcelona, Spain. As it is the aim of this conference to evaluate the status in all possible expression hosts, the organizing committee hopes to welcome also groups working in the area of recombinant protein production in plant cells as this field of research was sadly not represented in this year's conference. It would be a complementary addition to the wide range of topics discussed in this meeting series. Details regarding the RPP8 conference will be posted on the EFB website (www.efb-central.org).

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