

**1st FEBS Advanced Lecture Course on
Systems Biology:
From Molecules & Modeling to Cells
Gosau, Austria, EU, March 12-18, 2005**



Organizers

Anneke (J.G.) Koster (course director)
Institute for Systems Biology Amsterdam

Roland Eils
Intelligent Bioinformatics Systems
Deutsches KrebsForschungsZentrum
Heidelberg

Karl Kuchler
Department of Medical Biochemistry
Max F. Perutz Laboratories
Campus Vienna Biocenter
Medical University Vienna

Hans V. Westerhoff (program chair)
Departments of Molecular Cell Physiology and
Mathematical Biochemistry, BioCentrum
Amsterdam, Free University Amsterdam and
University of Amsterdam



U-P19 Evolutionary conservation and divergence of fungal promoter sequences

Riccarda Rischatsch, Sylvia Voegeli and Peter Philippsen

Applied Microbiology, Biozentrum Basel, Klingelbergstr. 50-70, Basel CH-4056, Switzerland, Phone: +41 61 267 14 89, FAX: +41 61 267 14 81, e-mail: Riccarda.Rischatsch@unibas.ch

The recently sequenced and fully annotated genome of the filamentous fungus *Ashbya gossypii* revealed striking similarity to the baker's yeast *Saccharomyces cerevisiae*. 90% of *A. gossypii* genes share homology and also a substantial degree of synteny (gene order conservation) with *S. cerevisiae*. Although both organisms originate from the same ancestor (carrying about 5000 protein coding genes), the evaluation of synteny was complicated by the fact that their evolutionary paths included not only about 300 translocations and inversions but also a whole genome duplication in the *S. cerevisiae* lineage followed by loss of 4000 genes. As a consequence the alignment of the *A. gossypii* genes with homologous *S. cerevisiae* genes results in many synteny clusters in which one *A. gossypii* chromosomal region aligns with two chromosomal regions of *S. cerevisiae*. The clusters themselves are made of gene regions displaying relaxed (incomplete) and stringent (complete) synteny. The latter is found in many small regions of up to eleven genes which, very importantly, are not interrupted by end points of rearrangements. Thus, these regions are particularly suitable for investigations of evolutionary conservation and divergence of syntenic sequences which started diverging over 100 million years ago. In the past, most studies of syntenic regions looked into conservation and divergence of open reading frames (ORFs) and the proteins they encode. We have started an investigation of evolutionary selection regarding size and sequence of inter-ORF regions. A detailed discussion of the subject will be presented taking into account DNA-binding sites of transcription factors, transcription start and terminator sites and inter-ORF lengths discerning between bidirectional or unidirectional promoters and pure terminator-bearing inter-ORFs.

U-P20 A Systems Biology approach for the optimization of recombinant protein production in *E. coli*

Eugénio Ferreira and Isabel Rocha

Dept. Eng. Biologica, Universidade do Minho, Campus de Gualtar, Braga 4710-057, Portugal, Phone: +351 253 604 407, FAX: +351 253 678 986, e-mail: ecferreira@deb.uminho.pt, Web: <http://www.deb.uminho.pt/ecferreira>

Escherichia coli has been the organism of choice for the production of many recombinant proteins with high therapeutic value. However, while the research on molecular biology has allowed the development of very strong promoters, there are still several phenomena associated with this process that have hampered the full use of that promoter strength, namely the aerobic acetate production associated with high specific growth rates. The presence of acetate is known to reduce both biomass yield on the chosen carbon source and protein productivity while totally inhibiting growth when present at high concentrations due to its toxic effect. While there have been several studies covering the recombinant protein production process with the bacterium *Escherichia coli*, including genome-scale analysis of the transcriptome, proteome, fluxome or metabolome, there has been a lack of an integrative approach that is able to combine genomic and physiological information about those processes with high-throughput analysis.

Also, the existence of genome-scale models that cover both stoichiometry and regulation of some pathways has not been taken into account in genome-scale data analysis and for the consequent formulation of hypothesis and development of new strategies for improving the performance of the process.

In our group, a high-cell density fed-batch process for recombinant protein production in *E. coli* is being studied, giving particular relevance to acetate production. A systematic approach is being used, by first compiling the existing knowledge about this phenomenon, extending existing genome-scale models to accommodate that knowledge, derive hypothesis in silico that are then tested by using genome-scale analysis of the omes. A reliable fermentation process was developed to be able to reproducibly study this phenomenon in different strains in order to reduce external variances to a minimum.