Projects for full-time Ph.D. studies within EST-project "Systems Biology"

Project 1

Title: Quantitative analysis and modelling of transcription initiation
Main supervisor: Roel van Driel
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Additional supervisors: Reinhart Heinrich, Stefan Hohmann

Aims

Extending the recently developed quantitative and predictive model for in vivo DNA nucleotide excision repair to transcription initiation.

Background

A few years ago our group has developed a novel method to analyse the detailed kinetics of the assembly of the chromatin-associated DNA repair (nucleotide excision repair) protein complex on damaged DNA in the nucleus of living mammalian cells (Moné et al., 2001; Moné et al., 2004)[1]. In this study we used a set of GFP-tagged DNA repair proteins and followed their assembly onto damaged DNA. In close cooperation with the group of Reinhart Heinrich (Humboldt University in Berlin) a quantitative and predictive model has been developed, based on systematic in vivo measurements (Politi et al., submitted). We are presently broadening the experimental basis of this model and testing specific predictions made by the model experimentally.
We propose to extend the study of the DNA repair system to transcription initiation and regulation. Several basic characteristics that have been found for DNA repair may also hold for the in vivo assembly of transcription initiation complexes on promoters. This makes it likely that a similar modelling approach can be used for transcription initiation as has been used for DNA repair. An exciting aspect of transcription initiation is its strict regulation. This will add a new important dimension to our experimental and modelling approach.
Studying regulation of transcription has links to signal transduction, creating a basis for cooperation with the Hohmann group.

Approach and expected results

A key aspect of DNA repair is that all DNA lesions are repaired in an essentially identical way, resulting in relatively high signal-to-noise ratios. In contrast, transcription is characterized by a different transcription initiation complex for each gene. To be able to measure the assembly of transcription complexes (and its control), an amplified system is required in which on at least many tens of genes identical initiation complexes are assembled.
Two ways are considered to achieve this. First, employ cells in which a specific gene has been tandemly amplified. Several of such cell lines have been described. Alternatively, assembly of at least part of the transcription initiation process can be mimicked by exploiting the initial steps of the DNA repair process. A founding transcription factor (e.g. the glucocorticoid receptor, which is able to initiate chromatin remodelling and transcription site assembly) can be fused to the damaged-DNA recognizing domain of protein XPC, the damage recognition factor in DNA repair. This allows triggering of the assembly of transcription initiation-like complexes by a brief exposure to UV and would ensure a strong signal of accumulation of GFP-tagged transcription factors. Systematic in vivo measurements will constitute the basis for a quantitative molecular model for transcription initiation, in cooperation with the Heinrich group.

Expected competence of Ph.D. student

The student should have a background in molecular biology or biochemistry.

[1] This work is part of a Dutch research program in cooperation with the group of Hoeijmakers, Vermeulen and Houtsmuller in Rotterdam and Mullenders and Van Zeeland in Leiden. This work is funded by the biological (ALW) and medical branch (ZonMW) of the Dutch Research Council NWO.

Formal conditions

Researchers must not be nationals of The Netherlands. At the start of their fellowship/activity, researchers may not have resided or carried out their main activity (work, studies, etc) in The Netherlands for more than 12 months in the 3 years immediately prior to the date of selection.

References

1. Moné, M.J., Volker, M., Nikaido, O. Mullenders, L.H.F., Van Zeeland, A.A., Verschure, P.J., Manders, E.M.M. and Van Driel, R. 2001. Local UV induced DNA damage in cell nuclei results in local transcription inhibition. *EMBO Reports*. 2:1013-1017.
2. Moné, M.J., Bernas, T., Dinant, C., Goedvree, F.A., and Manders, E. M., Volker, M., Houtsmuller, A.B., Hoeijmakers, J.H.J., Vermeulen, W. and Van Driel, R. 2004. In vivo dynamics of chromatin-associated complex formation in mammalian nucleotide excision repair. *Proc Natl Acad Sci U S A. in press*.
3. Politi A., Moné M.J., Houtsmuller A.B., Hoogstraten, D., Vermeulen, W., Heinrich, R. and Van Driel, R. Mathematical modeling of nucleotide excision repair reveals efficiency of sequential assembly strategies, *submitted*

Project 2

Title: Bioinformatics and modelling tools for genome-wide analysis
Main supervisor: Olle Nerman
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Additional supervisors: Edda Klipp, Per Sunnerhagen (via Stefan Hohmann)

Aims

To establish bioinformatics and modelling tools for genome-wide analysis of post-transcriptional regulation pathways, using comparative genomics as guiding principle.

Background

In a project, primarily involving cell biologists and bioinformaticians/ biostatisticians, aspects of eukaryotic post-transcriptional regulation will be studied. Post-transcriptional control is relevant primarily for rapid and reversible regulatory processes, such as stress responses. We will study several orthologous signalling pathways of species chosen from 18 fully sequenced yeast and other fungal species, representing a wide range of evolutionary distances.

Approach

The approach combines experiments, comparative genomic techniques for sequence and system comparisons, and measurement modelling. We will utilize the results to revise earlier system level models combining gene and protein regulation mechanisms of the studied signalling systems in *S. cerevisiae* and *Sz. pombe*. We will also compare and study evolutionary aspects of the post-transcriptional mechanisms and of the studied pathway systems. The comparative in silico part of the project mainly concerns cis-regulatory elements affecting the regulation of translation and gene mRNA degradation, and the experiments will mainly use specially designed RNA fractionation protocols combined with DNA arrays. The project extends several, local, national and international multi-disciplinary research and research training initiatives in genomics/bioinformatics and quantitative biology (including two EC projects) that we are involved in.
We expect to advance the fundamental understanding of genetic regulation at the mRNA level in eukaryotes and establish mathematical models for post-transcriptional regulation, and tools for cross-species conservation of regulatory patterns.

Expected competence of Ph.D. student

The Ph D student we wish to hire, should primarily work with the statistical, mathematical and computational modelling and analysis parts of the project, and he/she should ideally have a relevant specialisation on top of a broad training in Biotechnology, or vice versa a broad bioinformatics/biology training on top of a more theoretical basic university training.

Formal conditions

Researchers must not be nationals of Sweden. At the start of their fellowship/activity, researchers may not have resided or carried out their main activity (work, studies, etc) in Sweden for more than 12 months in the 3 years immediately prior to the date of selection.

Project 3

Title: Studying the aging process in yeast

Main supervisor: Edda Klipp
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Additional supervisors: Thomas Nyström (via Stefan Hohmann), Axel Kowald

Aims

Achieving a systems level understanding of the ageing process in yeast as a model system.

Background

Yeast cells have a genetically defined replicative life span. Aging is a complex process that is influenced by a series of external and internal factors. As a main cause of yeast aging the genetic instability of repeated, highly transcribed DNA is discussed (Sinclair and Guarente, 1997, Cell). Multiple factors influence this stability, including DNA repair processes, replication and metabolic activity. Another possibility is the accumulation of oxidized proteins, which could be diluted through cell division.

The combination of molecular biology, global expression measurements, and bioimaging on the one hand and dynamic modelling on the other hand will allow to rationalise observed phenomena and describe the process. Application to aging of multicellular organisms is envisaged.

Approach and expected results

In this project different possible cause of aging will be examined theoretically and experimentally. The first step for this is the development of a computational model, which examines the plausibility of the presented scenarios. In particular the influence of the size difference between mother and daughter for the division process will be analysed, since it is to be assumed that this parameter is of important influence for the number of possible divisions. Yeast mutants with different budding sizes exist (*cln1,2,3*, *cdc28*) and their behaviour will be considered in the study. In a later stage, the relation of the aging processes to the regulation of cell cycle processes will be investigated using integrated mathematical models. Experimentally, the size distribution can be measured with a high throughput flow cytometer. Then, cells are analysed with respect to their replicative potential in relation to size.

We expect to move forward the understanding of aging processes in yeast and the relation to the regulation of cell cycle processes. Although the conservation of metabolic and signalling pathways between yeast and humans is strikingly high, it is not necessarily the case that aging mechanisms will also be common to both organisms. But, since many of the genes that extend yeast life span have human counterparts it is likely that in both organisms, similar cellular systems will fail earlier that others.

Expected competence of Ph.D. student

The PhD student working on that project should have a background either in theoretical physics, biophysics, biochemistry or bioinfomatics.

Formal conditions

Researchers must not be nationals of Germany. At the start of their fellowship/activity, researchers may not have resided or carried out their main activity (work, studies, etc) in Germany for more than 12 months in the 3 years immediately prior to the date of selection.

References

1. Sinclair DA and Guarente L (1997) Extrachromosomal rDNA Circles - A Cause of Aging in Yeast, Cell, Vol. 91, 1033–1042,
2. Aguilaniu, H., Gustafsson, L., Rigoulet, M., and Nyström, T. (2003) Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. Science 299:1751-1753.
3. Hlavata, L. Aguilaniu, H. Pichova, A., and Nyström, T. (2003) The oncogenic Ras2val19 allele elevates ROS production and locks mitochondrial respiration in a non-phosphorylating mode independently of the PKA pathway. EMBO J. 22, 3337-3345.
4. Kofahl B. & Klipp E. 2004. Modeling the Dynamics of the Yeast Pheromone Pathway. *YEAST*, 21, 831-850.

Project 4

Title: Analysis of the evolutionary design of metabolic networks
Main supervisor: Reinhart Heinrich
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Additional supervisors: Hans Westerhoff, Barbara Bakker, Lena Gustafson

Background

In contrast to chemical reactions of inanimate nature the biochemical reaction networks within living cells are the outcome of natural selection during evolution. The project focuses on the theoretical elucidation of structural relationships in metabolic networks on a large scale by using a new method for simulating evolutionary processes. It is based on a network expansion starting from certain seed compounds and uses informations from databases for all enzymatic reactions taking place in metabolic networks.

Approach and expected results

The following problems have to be solved: 1) Determination of network modules, called scopes, being subsystems comprising all biochemical compounds which can be synthesized from a limited number of external substrates, (2) Analysis of the robustness of these modules with respect to deletions of certain reactions, (3) Construction of phylogenetic trees on the basis of metabolic data by taking into account the network structure of different species, and comparing the results with phylogenetic trees obtained from DNA sequence data, and (4) Deriving conclusions for the temporal order of the emergence of specific metabolic pathways during evolution. The project combines approaches from systems biology of metabolic networks, such as stoichiometric analysis and flux balance analysis, with approaches of bioinformatics, such as gene sequence analysis, working with databases, visualization of networks, and genetic algorithms. It is also aimed to extend the method of evolutionary network expansion to processes of intracellular signal transduction.

Expected competence of Ph.D. student

The PhD student working on that project should have a background either in theoretical physics, biophysics, biochemistry or bioinfomatics.

Formal conditions

Researchers must not be nationals of Germany. At the start of their fellowship/activity, researchers may not have resided or carried out their main activity (work, studies, etc) in Germany for more than 12 months in the 3 years immediately prior to the date of selection.

References

1. Ebenhöh, O., Handorf, T., and Heinrich R. (2004) Structural Analysis of Expanding Metabolic Networks. *Genome Informatics* **15**, 35-45.
2. Binder, B. and Heinrich R. (2004) Interrelations between dynamical properties and structural characteristics of signal transduction networks. *Genome Informatics* **15**, 13-23.
3. Lee, E., Salic, A., Krüger, R., Heinrich, R., and Kirschner, M. W. (2003) The roles of APC and axin derived from experimental and theoretical analysis of the Wnt pathway. *PLOS Biology* **1**, 116-132.
4. Heinrich, R., Neel, B.G., and Rapoport, T.A. (2002) Mathematical models of protein kinase signal transduction. *Mol. Cell* **9**, 957-970.

Project 5

Title: Quantitative biology of osmoregulation and volume dependence of cellular processes
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Additional supervisors: Edda Klipp

Aims

The aims are to achieve a better understanding of quantitative aspects of osmoregulation and the effect of cell volume on cellular processes.

Background

The control of volume, turgor and the relative intracellular water content are fundamental to life. We have previously investigated the response of yeast cells to osmotic stress and a model for the underlying processes has been generated. Now we wish to extend on this previous work and study osmoregulation at the single cell level. Emphasis will be on the effect of concentration changes due to altered cell volume on different cellular processes. Yeast will be used as a model system.

Approach and expected results

Experimentally there will be an emphasis on establishing and optimising tools to monitor cellular processes, specifically diffusion processes and volume regulation in collaboration with physicists and cell biologists. The project will employ tools of optical manipulation, microscopy, bioimaging and flow cytometry. Studies will make use of concentration dependent dyes and fluorescent agents as well as GFP (CFP, YFP, RFP). Cells will be treated by osmotic shock to reduce their cell volume and the consequences on reduced cell volume will be monitored at the level of individual cells, certain reporter proteins and genes as well as protein interactions. Cell recovery will be monitored at the level of cell volume as well as signal transduction and response mechanisms.

Experiments will be designed in collaboration with the theoretical group. The collaborating group will extend the theoretical framework for yeast volume and osmoregulation and the student will work at times together with theoreticians on modelling and simulation.

We expect a better understanding of osmoregulatory processes at the level of signal transduction and volume control, as well as tools and competence in studying these processes at the level of cell biology.

Expected competence of Ph.D. student

This is a project for students with a background in cell and molecular biology. Prior experience with microscopy and bioimaging would be an advantage. An interest in theoretical biology and biophysics (although not necessarily prior knowledge) will be required.

Formal conditions

Researchers must not be nationals of Sweden. At the start of their fellowship/activity, researchers may not have resided or carried out their main activity (work, studies, etc) in Sweden for more than 12 months in the 3 years immediately prior to the date of selection.

References

1. Klipp E, Nordlander B, Krüger R, Gennemark P, Hohmann S (2004) The dynamic response of yeast cells to osmotic shock – a systems biology approach. Submitted.
2. Hohmann S (2002) Osmotic stress signaling and osmoadaptation in yeast. Molecular Biology and Microbiology Reviews. 66: 300-372.