

Bela Novak, Jotun Hein, Chris Holmes and Steve Roberts
Case for Support EPSRC Grant Application November 2006

“Engineering Systems Biology of the Cell Cycle”

Part I: Previous Research Track Record

Principal Investigator: Professor Bela Novak

I will move to the Department of Biochemistry at Oxford University on 1 April 2007 in order to occupy the newly established Chair of Integrative Systems Biology. At the moment I work at the Budapest University of Technology and Economics where I am leading the Research Group of Molecular Network Dynamics financed by Hungarian Academy of Sciences. I have started my scientific carrier as an experimental biologist working in the field of fission yeast cell cycle (e.g. with Prof. Murdoch Mitchison in Edinburgh). When the molecular details of cell cycle control first started to emerge in the late 1980s, I became interested in connecting the molecular and physiological aspects of the controls. I have started a long and productive collaboration with Professor John J. Tyson (Virginia Tech, Blacksburg) to develop mathematical models of the cell cycle. I was convinced, and am still convinced, that a mathematical approach is required for deciphering the intricacies of biological systems. During the last 15 years we have developed realistic computational models for the cell cycle of (budding and fission) yeasts, early embryos and mammalian cells. Our models for the eukaryotic cell cycle are among the pioneering examples of the emerging field of systems biology.

Co-Investigator: Professor Steve Roberts

Prof. Roberts (<http://www.robots.ox.ac.uk/~sjrob/>) has extensive experience in applying signal processing and pattern recognition techniques to data, especially large, artefact-contaminated datasets. He is Professor in Information Engineering and heads the Pattern Analysis & Machine Learning Research Group, in the Department of Engineering at the University of Oxford. His group currently has 4 postdocs and 14 PhD students. Work in the group tackles the issue of data analysis from a theoretical perspective (that of Bayesian statistics) and has shown that a principled mathematical approach to the handling of uncertainty may be developed and applied to real-world problems, and systems based on this paradigm can dramatically improve results. Early funded projects (SERC, Wellcome Trust) developed techniques for advanced biomedical signal processing. Other funded projects include EEG signal analysis research (EPSRC 1996-1999 and BAE systems, 1996-2001), analysis of cancer evolution in HIV patients (Jefferiss Research Trust, 1998-2000), novel methods of unsupervised data analysis (EPSRC ROPA, 1999-2001), a large EU Framework IV project (1997-2000) in which the development of Bayesian methods for EEG signal processing and data analysis was led by our group. The EPSRC has funded (1996-1999) the theoretical development of techniques for learning and structure determination in Bayesian graphical models of bio-signal interaction and the BUPA Foundation awarded funding for machine-learning approaches to Brain-Computer Interfacing (2000-2003). Prof. Roberts was also a co-applicant on the large EPSRC/MRC interdisciplinary research centre (IRC) in medical signal and image processing (1999). Recently he has obtained funding from the EPSRC to research data analysis approaches to detect patterns of behaviour in bird flight and from BBSRC to investigate Bayesian approaches to gene micro-array analysis. The EPSRC, DTI and industry have recently (2003 & 2005) awarded very large grants to develop approaches in decentralised information fusion. Prof. Roberts has worked with industry on a variety of projects. Prof. Roberts has over 170 publications in the area of data analysis and machine learning, including editing two books.

Co-investigator Prof. Jotun Hein

I moved to the Department of Statistics at Oxford University in September 2001, coming from Aarhus University, where I was director of BiRC (Bioinformatics Research Center). Since I have come to Oxford I have worked on a series of issues. Most of my work is focused on developing methodologies that can analyze data arising in molecular genetics and genomics with a comparative/evolutionary focus. The work relevant to this proposal is especially Molecular Population Genetics and Comparative Genomics.

In *population genetics*, especially with Carsten Wiuf, Thomas Mailund and Mikkel Schierup from Aarhus University we work on the coalescent based methods of association mapping. We (Hein, Schierup and Wiuf, 2005) have published

a 300 page book on molecular population genetics titled "Gene Genealogies, Sequence Variation and Evolution" Oxford University Press, and are also involved in a very large EU collaboration (Holland, Denmark, Iceland and UK) to find susceptibility genes for breast and prostate cancer.

In *comparative genomics*, the most fundamental investigation is to find genes in a pair of aligned genomes. However, this can immediately be extended to many sequences, unaligned sequences, allowing for alternative splicing, RNA gene finding and a stronger focus on specifically motivated biological problems.

Bioinformatics is a field strongly in demand and we have contributed to the development of this field. Other research areas we are actively involved in include statistical alignment, RNA structure and protein coding constraints, haplotype block definition via reconstructed evolutionary histories and pathogen analysis. Besides being an active research group, we do much to increase awareness of this field in terms of public courses, seminars and organize lecture series.

Co-investigator: Dr Chris Holmes

I moved to Oxford to become a lecturer in Statistical Genetics in February 2004, having previously spent 3 years as a lecturer in Statistics in the department of Mathematics at Imperial College London. During and subsequently following my PhD in 1999 I have been working on Bayesian statistical modelling techniques for complex non-linear systems. My interests include methodological developments in Machine Learning and Stochastic Computation, in particular novel Markov Chain Monte Carlo algorithms.

I recently served for two years as Honorary Secretary of the Research Committee of the Royal Statistical Society (RSS), a post I held for two years. Previous to this in 2003 I was awarded the biennial Research Prize by the RSS for my work on Bayesian nonlinear regression models. I serve as Honorary Editor of the journal of Evolutionary Bioinformatics. I am co-organizer of a four week research program on "Bayesian nonparametric regression" to be held at the Isaac Newton Institute for Mathematical Sciences in Cambridge during July 2007.

My research group is based at the Oxford Centre for Gene Function and currently contains three postdocs and six DPhil students many of whom work on joint projects involving collaborations with experimental groups, including, investigators at the Pathogen Group in Zoology; the Wellcome Trust Centre for Human Genetics; the Centre for Statistics in Medicine and; the Broad Institute, MIT. My postdocs work on novel Bayesian methodology for pattern recognition models including non-linear methods and population based Markov Chain Monte Carlo algorithms for statistical computing. Of the six DPhil students, one works on new Bayesian approaches to mapping QTL jointly with Richard Mott at the WTCHG; one works on integrative genomics combining gene expression data with clinical markers for prognostic forecasting, joint with Doug Altman at the CSM; one works on novel Bayesian clustering models for de novo motif finding of regulatory elements; one works on models for SNP calling, in a joint project with the Broad Institute at MIT; one has just started working on copy number variants and their functional consequence in the mouse, and one has just started working on epidemiological models of sequence evolution in pathogens, incorporating space-time data in viral genealogies.

Case for Support BBSRC Grant Application November 2006

“Engineering Systems Biology of the Cell Cycle”

Part I: Research Proposal

A. Introduction of topic of research and its academic and wider context

The cell cycle - the sequence of events by which a cell grows, replicates its DNA, and divides into progeny cells - plays a crucial role in all biological growth, reproduction and development. The molecular machinery underlying the cell cycle is known to be highly conserved among all eukaryotes; not only the proteins that carry out such basic processes as DNA replication and mitosis, but also the molecular regulatory systems that control the timing of DNA synthesis and mitosis, the pace of cell division, and the response to proliferative and anti-proliferative signals (growth factors, DNA damage, nutritional status, etc.).

Major events of the eukaryotic cell cycle are controlled by a family of cyclin-dependent kinases (Cdk1, 2...) along with their cyclin partners (cyclin A, B...) and ancillary proteins that regulate the activities of Cdk-cyclin dimers. Using rigorous mathematical modeling, analysis and simulation, Novak & Tyson have studied the dynamical properties of this protein interaction network (the ‘cell cycle engine’) that drives the alternation of DNA replication and mitosis and that balances cell division to the rate of cell growth. Their models of budding and fission yeast cells, frog embryo and mammalian cells are successful in accounting for a broad spectrum of experimental results and in making unintuitive predictions that have been confirmed experimentally. However all of these models were developed on diverse molecular genetic data coming from very different type of experiments. Since molecular biology entered into a new exciting phase by developing high-throughput methods to collect data, it is timely to analyze these models in the framework of the new data sets which were not available at the time of model construction.

In this proposal we aim to explore an engineering framework for integrating information from high-throughput experimental data into system identification of well characterised mathematical models. We propose to do this using a Bayesian systems engineering approach. Our goal is to provide a statistically rigorous information engineering framework for constructing mathematical systems biology models using state space representations. Our approach will broadly consist of:

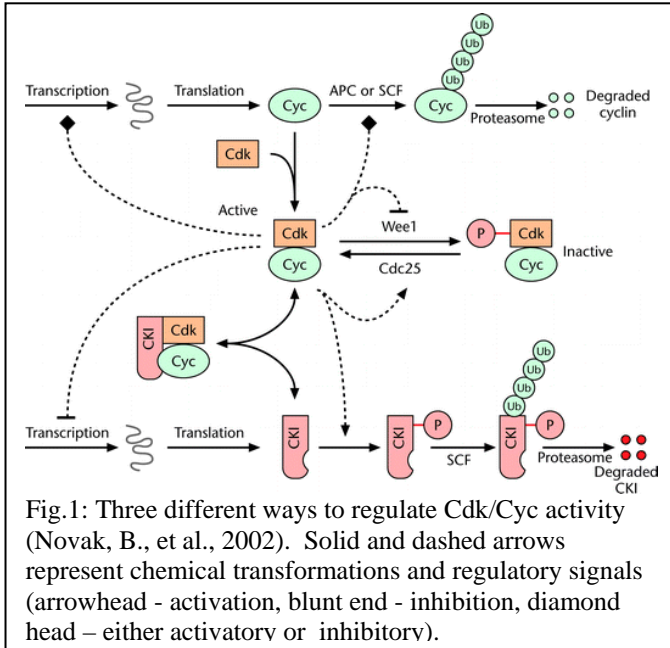
1. Develop a framework for mapping existing mathematical models into engineering systems dynamical models using state space representations of the kinetic parameters of the system with Gaussian process models of uncertainty on parameter rates.
2. Develop mathematical equations linking protein level models with transcription level models and derive the appropriate likelihoods (observation equation sampling distributions).
3. Develop strategies for incorporating background information, from literature or related studies, into prior probability distributions on plausible settings of rate parameters within the models.
4. Develop stochastic simulation methods, using particle filters and sequential Monte Carlo to be able to make inference on kinetic rate parameters and generate predictive stochastic trajectories from the models.
5. Develop methods of structural systems identification, to help elicit putative interactions within the neighbourhood of the existing models, and to quantify the evidence of existing interactions.

We will develop our framework in the context of yeast cell cycle data. Both budding (*Saccharomyces cerevisiae*) and fission (*Schizosaccharomyces pombe*) yeast are extremely well suited for this type of study. In particular, their genome sequence is completely mapped out and genome-wide studies have yielded useful information about each individual gene: including, their pattern of transcription, and the abundance and localization of their protein products at various stages of the cell cycle. We will use the publicly available genome-wide gene expression data of budding and fission yeast cell cycles to test bed our approach.

Broadly speaking, our approach is akin to system identification of rate parameters under rigorous constraints. The form of the underlying cellular dynamics leads us to systems which are *partially observed* and *partially known* in the sense that biochemical insight and previous mathematical modelling leads to a set of coupled equations, the control parameters of which cannot all be inferred from the data. The combination of such *deep domain knowledge* and

empirical inference from data using joint domain and *system identification* models has been effective in the past (see for example, Kar et al 1999). One of the key constraints we can use lies in the fact that we believe *a priori* that the cellular dynamics is *stable* which restricts the parameter inference and enhances tractability.

B. The Yeast Cell Cycle.



mediated proteolysis), by reversible inhibitory tyrosine-phosphorylation of Cdk1 (by Wee1-type kinases and Cdc25-type phosphatases), and by binding of stoichiometric Cdk1 inhibitors (CKI). These regulatory processes are modulated by the very Cdk1/CycB complexes they control, thereby creating **feedback loops** in the mechanism (Fig. 1). The most important difference between cell cycle regulation in the two yeasts is that inhibitory Tyr-phosphorylation of Cdk1 subunit, which is responsible for the long G2 phase of fission yeast cell cycle, is not significant in the budding yeast.

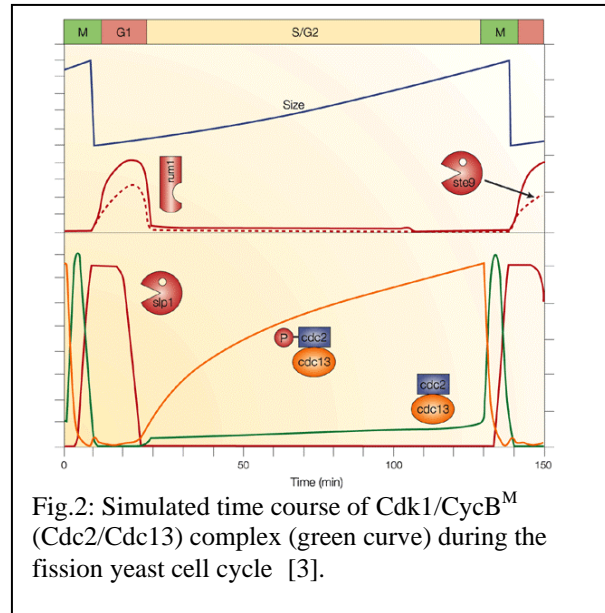
C. Mathematical modelling of the cell cycle

From this complex web of regulatory interactions among cell cycle regulators, the PI has constructed mathematical models of cell cycle controls for many different eukaryotic organisms (from embryos through yeasts to mammalian cells (Novak, B and Tyson, J.J., 1993; Novak, B. and Tyson, J.J., 2004). The control system is converted by the standard rules of biochemical kinetics into a set of differential equations. For instance, the time rate of change of Cdk/Cyc on Fig.1 can be described as follows:

$$d/dt [\text{Cdk/Cyc}] = k_s[\text{Cdk}][\text{Cyc}] - k_a[\text{Cdk/Cyc}][\text{CKI}] + k_d[\text{CKI/Cdk/Cyc}] - k_w[\text{Wee1}][\text{Cdk/Cyc}] + k_{25}[\text{Cdc25}][\text{P-Cdk/Cyc}]$$

The cell division cycle is the periodic repetition of a sequence of events - including DNA replication (S phase), mitosis (M phase), and cytokinesis - that transform a single cell into two daughter cells (Morgan, D.O., 2006). Progression through the eukaryotic cell cycle is driven by a conserved network of proteins, centred upon Cdk's (Cyclin dependent protein-kinase) and their regulatory subunits (cyclins). In simple eukaryotes, like yeasts there is only one essential Cdk subunit (Cdk1) which forms complexes with two different types of B-type cyclins (CycB). The complex of Cdk1 with S phase cyclin (CycB^S) and M phase cyclin (CycB^M) triggers DNA replication and mitosis. Therefore the activities of Cdk1/CycB^S and Cdk1/CycB^M complexes show alternating oscillations peaking during S and M phases of the yeasts cell cycle.

In general, the activity of Cdk1/CycB complexes could be regulated by multiple mechanisms (Novak, B. et al. 2002) by availability of cyclin partners (via transcriptional control and/or regulated ubiquitin



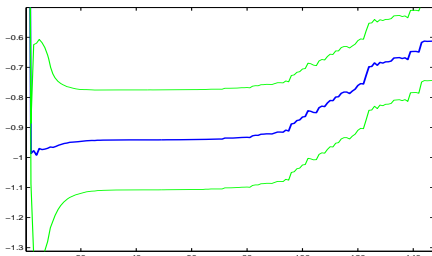
and similar equations can be written for all the other components in the network. These equations can be solved numerically **after specifying the parameter values** (rate constants etc.) and a representative simulation of the fission yeast cell cycle is shown in Fig.2.

However, a realistic model of a yeast cell cycle control involves dozens of kinetic parameters and only few of these can be determined directly from well-designed experiments. We previously used bifurcation theory that tells us how the generic properties of a dynamical system depend on parameter values. Although this method of parameter estimation of cell cycle models provided satisfactory results, it relies only on physiological characteristics of mutants created by classical or molecular genetic methods. However the method is completely ignorant with recent high throughput data coming from top-down approaches of systems biology. In the framework of this proposal we would like to integrate into the cell cycle models the data coming from large scale analysis of the yeast's transcriptome and proteome (see Data section below).

Systems Identification: Statistical Inference of Rate Parameters and Structural Forms.

The existing mathematical models assume the solutions are those of feedback driven *first order* differential equations. In this case we propose to build simulators based on Gaussian processes for the *state space* variables x and dx/dt , as is common in control analysis. Taking the Gaussian process kernel to be smooth, we may impute curves for dx/dt , which are "centred" on the current mathematical equations, and then integrate the covariance functions to define Gaussian equations for the observation process. The effect of different control parameters becomes clear if we draw samples from a Gaussian process over the derivative and calculate the solution.

We will initially discretise the model in order to form a delay-difference proxy of the differential equations in continuous time. This enables marginal likelihoods over latent or partially observed data to be inferred and for uncertainty to be integrated out of the final imputation. Fully Bayesian extensions of these models are in the literature [Roberts & Penny, 2002; Penny & Roberts, 2002, for example]. We then will consider continuous time models with diffusion processes.



Previous work using a fully Bayesian dynamical model (based on coupled first order differential equations) is presented in [Kar *et al.* 2004] in which a sequential Bayes model is used to infer rate parameters and hence reconstruct observation processes. The figure to the left shows a simple simulation in which the non-constant rate parameter $a[t]$ in the differential Equation $dy/dt = -a[t]y$ is inferred using a Bayes Kalman filter based on noisy samples of the system (30% noise by amplitude). The parameter had a linear drift which is clearly picked up by the approach. More importantly, full predictive densities are obtained which enables subsequent integration over uncertainty. Error bars

are shown at +/- 2SD.

The systems models of the cell cycle process will be very complex. A major research exercise will be to assess whether such Gaussian process representations of systems derivatives (that is, modelling the coupled derivatives of the system as a time-inhomogeneous Gaussian processes centred on the current mathematical descriptions) will provide a useful inference framework in systems biology; and in particular for the fusion of mathematical models with experimental data. Complicating issues include, non-stationary covariance functions, non-Gaussian likelihoods, missing data and data with nonstationary error.

However, if we are able to perform this first stage then it will allow us to incorporate prior knowledge and uncertainty directly into the engineering model; so we might use information from related studies to help specify a plausible range of parameter values, and we will have the foundation for systems identification in systems biology models.

Likelihoods: The mathematical models developed previously by the PI are defined on the protein level, while most of our experimental data will arise from measurements of mRNA transcription levels (see Data section below). The elicitation of a dynamic likelihood function linking the unobserved state of the system at the protein level to a probability distribution on observed mRNA levels will be a major part of the early research phase. This will involve the construction of dynamical systems models linking expected levels of protein and RNA as well as empirical

quantification of residual uncertainty in the data: and whether this can be realistically assumed, or transformed, into Gaussian form.

The initial task is to develop models with differential equations relating protein to transcript levels. These ODE's will have two terms on their right hand side: production term by the TF's and a decay term for degradation of the mRNA (e.g. first order kinetics):

$$\frac{d [\text{mRNA}]}{dt} = k_s \cdot \text{TF} - k_d \cdot [\text{mRNA}]$$

The solution of DE's for the mRNA's is then directly comparable with an experimental data set of gene expression. The activity change of the TF's involved in the transcription of cell cycle regulators will be described by separate DE's:

$$\frac{d [\text{TF}]}{dt} = - \frac{ki \cdot [\text{TF}]}{Ji + [\text{TF}]}$$

where $(1 - [\text{TF}])$ represent the inactive form of TF (assuming that the total concentration equals to one). Of course, the rate of change in the level of transcriptionally regulated proteins will be modified compared to the original models. As a consequence of these relationships the DE's for transcript and protein levels, and activities of TF's and proteins will form a closed set of equations that can be used for parameter estimation via the derivation of a likelihood function.

Non-Gaussian Likelihoods: For some low level RNAs, such as those related to transcription factors, we are unable to assume Gaussian likelihoods for the final observation equations governing the experimental data. A more appropriate sampling distribution would be a Gamma or Poisson. The adoption of non-Gaussian likelihoods significantly complicates the inference as many of the conditional distributions of the rate parameters will no longer be of closed form. This will necessitate advanced stochastic simulation methods.

Stochastic Simulation: We aim to develop Monte Carlo methods to perform inference and allow for simulation of predicted trajectories from our models. Particle filters and Sequential Monte Carlo methods provide a good starting point for our needs. These methods have been shown to work well on complex dynamic inference problems. However in complex continuous time non-Gaussian systems with feedback, as in our case, it is certain that we will need to tailor existing methods. Some recent work on dynamic re-weighting for sequential MC paths looks like a promising approach [Jasra, A., et al., 2006].

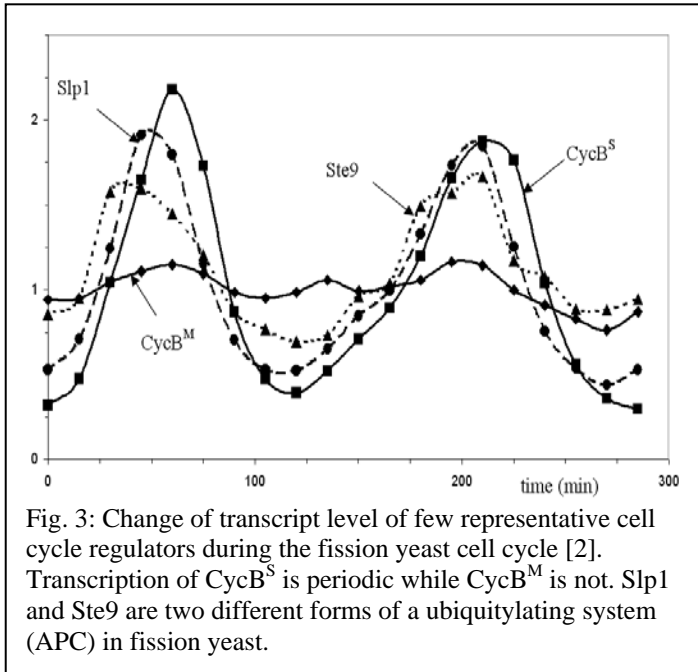
Model Comparison: Formal hypotheses testing of competing models will allow us to see whether the experimental data supports one description (model) for the data or a competing explanation (model). In our Bayesian framework, we will need to compute the *Bayes Factors*, ratio of marginal likelihoods. The problem is that we need numerical methods to calculate the necessary integrals over the parameter space. Conventional methods using thermodynamic integration are known to be subject to high variability and can provide poor approximation and it is unclear whether other approximate Bayesian techniques, such as variational learning will not work well due to the highly dependent posterior distributions induced by feedback. We aim to explore a number of recent algorithms published in the Physics literature, including nested sampling and multicanonical Monte Carlo.

D. Data.

Cell cycle control of transcription is a fundamental characteristic for the cell cycle of growing cells. Genome-wide studies of transcription during the cell cycle have identified hundreds of periodically expressed genes in different organisms. Many cell cycle regulator genes have periodic transcription (Fig 3) but periodicity extends beyond them. Since cell cycle periodic transcription is coordinated with overall cell cycle progression, the quantitative measure of transcript levels over the cell cycle should provide valuable data for parameterization of cell cycle models.

In the framework of the present proposal we would like to use the publicly available genome-wide gene expression data of budding and fission yeast cell cycles. There are already more than one independent studies in the literature about cell cycle periodic transcription and fission yeast. In these experiments different synchronization methods (selection and

induction synchrony) were used and the relative abundance of transcript levels were measured over few cell cycles (see e.g. Fig. 3).



Comparative analysis of the different datasets identifies about 600 and 500 periodically expressed genes in budding and fission yeast respectively. Periodically transcribed genes are not well conserved between these two evolutionary distantly related yeasts. Only about 40 orthologous genes are periodic in both organisms and all of them have a role in cell cycle progression. We will analyze the mRNA levels of those genes only which are represented in the yeasts cell cycle model. The differences between the two yeasts will provide important details for a comparative analysis. Quantitative measurements on protein levels of cell cycle regulators are much sparser. For most of the periodic cell cycle regulatory proteins the only information available are the point in the cell cycle when they start to accumulate and disappear. Hopefully this situation will change because of the TAP-tagged library makes it feasible to monitor the yeast proteome, and we will incorporate such data if it becomes available.

Phase II: Extensions and Comparative Aspects.

Conditional on the success of the first 18 months, the Oxford Centre for Systems Biology has agreed to fund the continuation of the project and hire for a total of 36 so that the initial projects will be extended and exploited. The basic dynamic models will focus on incorporating new data and knowledge (as of Oct.2008) and Stochastic phenomena due to some of the cell cycle regulators present at low copy number, the cell cycle engine has stochastic components. Stochasticity in cell cycle control causes a difference in cycle time of individual cells. We will develop stochastic models for the yeasts cell cycles based on the already existing deterministic ones.

The comparative investigations will have 2 components:

(i). At present the dynamical systems describing the cell cycle in for instance budding and fission yeast are two separate systems, while clearly these mechanisms are homologous. The inference of parameters in these systems should be tied to each other by an evolutionary mechanism allowing parameters to evolve and at a low frequency, reactions to be lost or recruited to the central mechanism. We will aim to define coupled sets of states space models for the cell cycles where the coupling strength is defined via an evolutionary distance. This appears a challenging engineering task, but if successful it would allow us to do joint systems identification (combining data and borrowing strength from both systems) to allow for more precise inference of rate parameters.

(ii). Comparative Genomics prediction:

If stage (i) is successful it will allow for prediction of how the central mechanism will appear in other yeast species, but where corresponding data and experiments and data is limited or not available; simply by knowledge of the genes and prediction of their function. This will allow for extension of the knowledge of the well studied species to the less well studies species by advanced use of homology.

Knowledge Transfer between Engineering and the Life Sciences

The post-holders will be based in the new Systems Biology Centre in the department of Biochemistry. The technical nature of the project and the CIs positions will lead to strong interactions with the Information Engineering group of Steve Roberts. We will request the post holders to organize three yearly (once per term) joint seminar meetings between Engineering and Biochemistry. This will provide direct dissemination of the results from the project as well as wider links between the groups. At these meetings we will invite an external speaker from outside of Oxford either from the Life Sciences or Information Engineering (so knowledge is also transferred through this route). We have also highlighted attendance at both Engineering and Biological conferences where we aim to disseminate our results.

G Timeliness

The large amounts of data from a series of throughput technologies coupled to mathematical and computer intensive statistical modelling will allow predictive modelling to be inferred for well-defined biological systems. We believe this kind of ambitious integrative systems modelling approach will dominate systems biology in the coming years.

H Milestones and management of the project

The 4 applicants will meet weekly with the two RAs to monitor progress and join in with the lab meetings.

Year 1-Months 1-3: RAs reads biological literature and collect relevant existing programs for yeast cell cycle modelling.

Year 1 Months 4-6: RAs will make simple simulation models that can generate artificial data involving all facets of the analysis. This is necessarily simplistic, as full details of complex aspects model versus data are unknown, but can become increasingly realistic as the project proceeds.

Year 1 Months 7-12: Inference of dynamic models from simulated data and evaluation of robustness and power of proposed methods.

Year 2 Months 13-18: Inference of dynamic models from real data. Publication activity and making status of Phase 1.

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