Biotechnology Advances 27 (2009) 960–978

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Systems biology of the cell cycle of *Saccharomyces cerevisiae*: From network mining to system-level properties

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ARTICLE INFO

Available online 22 May 2009

Keywords:
Systems biology
Cell cycle
G1/S transition
Cki
Sic1
Far1
Cln3
CK2
Mitotic exit

ABSTRACT

Following a brief description of the operational procedures of systems biology (SB), the cell cycle of budding yeast is discussed as a successful example of a top-down SB analysis. After the reconstruction of the steps that have led to the identification of a sizer plus timer network in the G1 to S transition, it is shown that basic functions of the cell cycle (the setting of the critical cell size and the accuracy of DNA replication) are system-level properties, detected only by integrating molecular analysis with modelling and simulation of their underlying networks. A detailed network structure of a second relevant regulatory step of the cell cycle, the exit from mitosis, derived from extensive data mining, is constructed and discussed.

To reach a quantitative understanding of how nutrients control, through signalling, metabolism and transcription, cell growth and cycle is a very relevant aim of SB. Since we know that about 900 gene products are required for cell cycle execution and control in budding yeast, it is quite clear that a purely systematic approach would require too much time. Therefore lines for a modular SB approach, which prioritises molecular and computational investigations for faster cell cycle understanding, are proposed.

The relevance of the insight coming from the cell cycle SB studies in developing a new framework for tackling very complex biological processes, such as cancer and aging, is discussed.

Contents

Abbreviations: APC, Anaphase Promoting Complex; Cdk1, Cyclin dependent kinase1 (Cdc28); CK2, Casein Kinase 2; FEAR, Cdc Fourteen Early Anaphase Release; GAP, GTPase activating protein; GEF, Guanine Exchange Factor; MBF, MCB, MluI Cell Cycle Box, Binding Factor; MCC, Mitotic Checkpoint Complex; MDT, Mass Duplication Time; MEN, Mitotic Exit Network; PKA, Protein Kinase A; PP2A, Protein Phosphatase 2A; SBF, SCB, Swi4/6 Cell Cycle Box, Binding Factor; SCF, Skp1-Cullin-F-box complexes; SPB, Spindle Pole Body.

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0734-9750/$ – see front matter
doi:10.1016/j.biotechadv.2009.05.021

1. Introduction

Biotechnology has so far been able to tackle significant, but relatively simple tasks such as the production, by recombinant DNA techniques, of human proteins for the treatment of diseases that are caused by reduced endogenous production (for example, insulin for diabetics, growth hormone for nanism, etc.); the development, by metabolic engineering techniques, of fermentation processes for the production of a large set of chemicals (for instance lactic acid, glycerol, ethanol, succinic acid and citric acid) (Bianchi et al., 2001; Wang, et al., 2001a; Kuyper et al., 2005; Lee et al., 2005; Berovic and Legisa, 2007); the production of monoclonal antibodies to be used to inactivate specific signalling pathways whose stimulation is thought to be responsible for a given disease (for instance, Trastuzumab/Herceptin and the recombinant humanised monoclonal antibody which targets the receptor HER-2, whose overexpression is involved in the pathogenesis of breast cancer) (Carter et al., 1992). But, in the particular case of Herceptin, a limit of this biotechnological approach has become apparent: the role of overexpressed HER-2 in sustaining neoplastic growth is more complex than anticipated. In fact, HER-2 acts both as an activator of signalling (response that is inhibited by the antibody) and a glucose transporter (that is not inhibited by the drug) (Weihua et al., 2008), thereby offering an explanation to the limited success of Herceptin in clinical trials, with the response rate between 10% and 20% only across a variety of human cancers (Fukouka et al., 2002; Kris et al., 2002; Cohen et al., 2003, Dancey and Freidlin, 2003).

The idea is therefore gaining ground that, in order to make drugs more effective against diseases that affect complex biological processes (such as cancer, neurodegenerative and metabolic diseases) or to gain more efficient control of bioprocesses (Christensen et al., 2009), a change of paradigm in biological research is needed, to be able to understand better and predict the dynamics of the complex biological functions (such as signalling, transcription, metabolism, cell cycle, cell death) (Hood and Perlmutter, 2004; Aderem, 2005; Henney and Superti-Furga, 2008).

The change of the required paradigm could be given by systems biology (Morohashi et al., 2002; Kitano, 2004a,b;). Why? Systems biology recognizes that only very rarely a given biological function strictly depends upon on a single gene product, but rather it is generated by the dynamic interaction of hundreds or thousands of gene products (Westerhoff and Alberghina, 2005). It has to be further stressed that the function is not going to derive by a sort of addition of the activities of the various gene products, but it is generated as an *emergent property* by their interactions structured in a network, perhaps modulated by small molecules derived from the environment (Westerhoff and Alberghina, 2005). Emergent (or system-level) properties are quite common in technological systems from which systems biology derives many concepts and methods (Hartwell et al., 1999). In order to be able to analyze the dynamics of a given network and also to identify its emergent properties, systems biology relies on an integrated and interdisciplinary approach of molecular analysis, often genome-level, and mathematical modelling and simulations as well (Westerhoff and Alberghina 2005).

Although the molecular composition of cells and organisms is of staggering complexity, a systems biology approach is made feasible by the fact that complex biological processes may be disassembled into modules and subsystems of interacting DNA, RNA, proteins and small molecules that perform a given task in a way that is substantially independent from the context (Hartwell et al., 1999). Signalling and metabolic pathways, transcriptional regulatory network, cell cycle and apoptosis are examples of modules that can be dissected in molecular terms and described by mathematical models independently one from the other (Bentele et al., 2004; Chen et al., 2004; Birtwistle et al., 2007; Mo and Palsson, 2009). Considering that modularity is organised by the fact that the output of a module is often the input of the following one (for instance, pyruvate is the output of glycolysis and the input of mitochondrial metabolic network), by global connectors among modules (for example, feedback and parallel interconnections) and that "party-hubs" connect the partners of each modules (Han et al., 2004), it is possible to assemble validated mathematical models of each module with the final aim, for instance, of constructing the model of an entire cell.

Another very relevant system-level property is robustness: biological networks are robust, since they are mostly able to maintain their function despite external and internal perturbations (Kitano, 2004a). Several design principles able to provide robustness have been described: feedback, redundancy, diversity, modularity and decoupling (Kitano, 2004a). Molecular identifications of several robustness devices are already available for many types of control. For instance, the best studied control system relies on a combination of positive and negative feedbacks to attain a robust dynamic response observed in several networks, including cell cycle, the circadian clock and chemotaxis (Alon et al., 1999; Morohashi et al., 2002). Bacterial chemotaxis is one of the best studied examples of robust adaptation that uses negative feedback to allow response to take place following a wide range of stimuli (Alon et al., 1999). Another example is given by tumour cells that turn on the expression of the multidrug resistance 1 gene (*MDR1*) acquiring multidrug resistance by exporting drugs out of the cell through an ATP-dependent efflux pump, which is encoded by *MDR1* (Juliano and Ling, 1976; Nooter and Herweijer, 1991). Thereby, this simple and very effective feedback-control mechanism minimizes cytotoxic levels, that, of course, hampers drug treatments. Therefore, as it is often said, a better understanding of robustness could facilitate more efficient drug discovery (Kitano, 2004b).

From where do we start in making models of biological processes? First of all we should recall that a model is only a symbolic representation of reality, which is able to foster our understanding and support decision-making, and that a mathematical model should be able to give a quantitative representation of a process and to make predictions. On the other hand, given that a system is a regularly interacting group of items forming a whole that behaves like a unit for a given performance, the knowledge of the function expressed by the system is central in developing a systems biology approach. When all the molecular constituents of a module are known, like the various steps of glycolysis, one may start from the molecular details and build up a model (*bottom-up*) to explain its physiological functions (for instance the Pasteur effect). But, when all the components are not known or too many (introducing therefore the possibility that their interactions generate so large a number of putative network structures, which could be impossible to discriminate the correct one amongst the rest by the available experimental approaches), it is a much wiser strategy to start modelling *top-down*. In this way one can move from the physiological function to reconstruct the underlying molecular mechanisms (Noble, 2002; Ingolia and Murray, 2004; Alberghina et al., 2005).

Block diagrams are used to dissect a cellular process in modules and to identify the governing interactions among them (positive or negative feedbacks, threshold control, amplification, etc.). Mathematical models and simulation analysis will allow us to evaluate whether the very basic map constructed in this way is able to capture the essential features of the process. If so, the components of a module and their interactions can be identified following an iterative process, in which (genetic and/or nutritional) perturbations of the cellular system, followed by cycles of molecular analysis (often genome-wide), modelling and simulation tests, will lead to a model that is detailed enough to be able to account for the behaviour of the system under investigation. This approach has been applied by our laboratory to increase our understanding of relevant steps of cell cycle control (Alberghina et al., 2001, 2004a; Rossi et al., 2005; Barberis et al., 2007).

2. Cell cycle control

Cell cycle is a very central process in living organisms since it stays at the basis of their fundamental property: the ability to grow and