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Cell growth and cell cycle in *Saccharomyces cerevisiae*: Basic regulatory design and protein–protein interaction network

Lilia Alberghina [a,*], Gabriella Mavelli [b,*], Guido Drovandi [b], Pasquale Palumbo [b], Stefania Pessina [a], Farida Tripodi [a], Paola Coccetti [a], Marco Vanoni [a,*]

[a] Dipartimento di Biotecnologie e Bioscienze, Università di Milano-Bicocca, Piazza della Scienza 2, 20126 Milano, Italy
[b] Istituto di Analisi dei Sistemi ed Informatica "Antonio Ruberti", Consiglio Nazionale delle Ricerche, Viale Manzoni 30, 00185 Roma, Italy

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ABSTRACT

In this review we summarize the major connections between cell growth and cell cycle in the model eukaryote *Saccharomyces cerevisiae*. In *S. cerevisiae* regulation of cell cycle progression is achieved predominantly during a narrow interval in the late G1 phase known as START (Pringle and Hartwell, 1981). At START a yeast cell integrates environmental and internal signals (such as nutrient availability, presence of pheromone, attainment of a critical size, status of the metabolic machinery) and decides whether to enter a new cell cycle or to undertake an alternative developmental program. Several signaling pathways, that act to connect the nutritional status to cellular actions, are briefly outlined. A *Growth & Cycle* interaction network has been manually curated. More than one fifth of the edges within the *Growth & Cycle* network connect *Growth* and *Cycle* proteins, indicating a strong interconnection between the processes of cell growth and cell cycle. The backbone of the *Growth & Cycle* network *is* composed of middle-degree nodes suggesting that it shares some properties with HOT networks.

The development of multi-scale modeling and simulation analysis will help to elucidate relevant central features of growth and cycle as well as to identify their system-level properties. Confident collaborative efforts involving different expertises will allow to construct consensus, integrated models effectively linking the processes of cell growth and cell cycle, ultimately contributing to shed more light also on diseases in which an altered proliferation ability is observed, such as cancer.

1. Introduction

Understanding complex cellular processes is a major challenge facing present-day biology. Reductionistic approaches that culminated with the complete sequencing of the human genome (Lander et al., 2001; Venter et al., 2001) allowed to collect the inventory of the informational molecules – from DNA to microRNA involved in the control of gene expression – that underlay any vital function. Concurrently, high throughput techniques (collectively referred to as *omics* technologies) have been developed to measure, in high throughput manner and with ever increasing precision, individual biochemical parameters (RNA, protein, metabolite content, metabolic fluxes, etc.) on a genome-wide, or close to genome-wide scale. This ever increasing information wealth gives to scientists an unprecedented analytical power, but by itself is not sufficient to bring understanding on the regulation of major complex biological processes at molecular level.

A new paradigm is needed, able to integrate molecular analysis with an approach that describes dynamic interactions among components of a living system so to correctly predict its behavior under changing circumstances (Hartwell et al., 1999; Nurse and Hayles, 2011). This new approach is offered by systems biology, characterized by the fact that molecular analysis is integrated, in iterative cycles, with computational modeling, simulation analysis and control theory (Alberghina and Westerhoff, 2005; Kitano, 2002; Westerhoff and Palsson, 2004). In a systems biology approach, biological processes are taken to be the results of complex, coordinated, dynamic, non-linear interactions of a large number of components, which are shaped by time and space constrains. These dynamic interactions generate, as emergent property of the system, the corresponding function, that therefore is not found in individual components, but only in their networking (Bhalla and Iyengar, 1999). Besides, a number of different molecular devices are able to confer robustness to biological function perturbed by external or internal cues (Kitano, 2007, 2010a; Stelling et al., 2004).

Abbreviations: AMPK, AMP-activated kinase; APC, Anaphase Promoting Complex; CK2, Casein Kinase 2; Cdk1, Cyclin-Dependent Kinase 1 (Cdc28); FEAR, Cdc Fourteen Early Anaphase Release; MDT, Mass Duplication Time; MEN, Mitotic Exit Network; PKA, Protein Kinase A; MBF, MCB Binding Factor; MCB, MluI Cell Cycle Box; PP2A, Protein Phosphatase 2A; SBF, SCB Binding Factor; SCB, Swi4/6 Cell Cycle Box; PPI, protein–protein interaction; PIN, Protein Interaction network.

* Corresponding authors: Tel.: +39 026448 3515; fax: +39 0264483519.
E-mail addresses: lilia.alberghina@unimib.it (L. Alberghina), gabriella.mavelli@iasi.cnr.it (G. Mavelli), marco.vanoni@unimib.it (M. Vanoni).

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When dealing with biological systems of increasing complexity, a modular approach is required for a successful systems biology analysis. Very briefly, modular systems biology investigation starts from the recognition that cellular processes can be disassembled into modules, subsystems of interacting molecules (proteins, DNA, RNA, and small molecules) that perform a given task in a way largely independent from the context (Alberghina et al., 2005). A given process can then be described by a blueprint in which its basic modules and their regulatory interactions (positive and negative feedbacks, threshold control, amplification, error correction, etc.) are represented (Alberghina et al., 2001). Modeling and simulation analysis will evaluate whether the very basic blueprint is able to capture essential performance of the process under investigation. If so, the components of each module and their molecular interactions can be identified by molecular analysis of all putative regulatory and effector components under steady and perturbed growth conditions. Iterative rounds of molecular analysis and of model building (Kitano, 2002) should allow achieving a satisfactory molecular model able to quantitatively account for the entire process.

Growth and cycle are among the more basic complex cellular functions that are expected to reach a deeper understanding through systems biology, so to shed more light also on diseases in which an altered proliferation ability is observed, as in cancer. As in many previous instances in biological research, it is convenient to start investigation on a model organism, the budding yeast *Saccharomyces cerevisiae*.

1.1. Saccharomyces cerevisiae as a model organism for the study of cell growth and cycle

The term growth is generally used to indicate both the increase in cell mass of an individual cell as well as the increase in number of a cell population. Cellular growth (mostly given by synthesis of ribosomes and proteins) proceeds continuously during the cycle (Alberghina and Porro, 1993; Elliott and McLaughlin, 1978; see also paragraph 2), while discontinuous events take place only once during a cell's lifetime (i.e., from its birth to its next division); these events include genome replication (S phase), mitosis and cell division (M), so that the eukaryotic cell cycle is divided in into four phases: G1 (gap1), S (synthesis of DNA), G2 (gap 2) and M. During the S phase the chromosomal DNA is replicated with great accuracy, while during the G1 and G2 phases events take place to respectively prepare and control the onset of DNA replication and then of mitosis.

The budding yeast *S. cerevisiae* is a simple and genetically tractable eukaryotic model organism used in many pioneering efforts, such as recombinant DNA techniques, systematic sequencing of genome, construction and analysis of gene deletion mutants or definition of the interactome (reviewed in Snyder and Gallagher, 2009). Besides, it has been used at length as a model for the study of eukaryotic cell growth and division as well as of metabolism and signaling.

As pointed out as early as in 1971 (Mitchison, 1971), the "continuous events of the growth cycle" (i.e., increase in cell mass) and the "discontinuous events of the DNA division cycle" (i.e., DNA replication, mitosis, and cell division) need to be tightly coordinated in order to maintain cell size homeostasis, thus preventing cells from becoming too small or too large. Nutrients are the main environmental determinants, with temperature (Vanoni et al., 1984) and mating factors (Cross and McKinney, 1992), that affect growth and cell cycle progression in budding yeast (Busti et al., 2010; Gutteridge et al., 2010; Lord and Wheals, 1980; Searle and Sanchez, 2004; Vanoni et al., 1983).

In *S. cerevisiae* regulation of cell cycle progression is achieved predominantly during a narrow interval in the late G1 phase known as START (Pringle and Hartwell, 1981). At START a yeast cell integrates environmental and internal signals (such as nutrient availability, presence of pheromone, attainment of a critical size, status of the metabolic machinery) and decides whether to enter a new cell cycle or to undertake an alternative developmental program (sporulation, conjugation, entry into stationary phase) (Pringle and Hartwell, 1981; Smets et al., 2010). Execution of START irreversibly commits the cell to a new mitotic cycle and requires the activation of Cdk1 (Cdc28). This cyclin-dependent kinase governs the major cell cycle transitions in budding yeast, its activity being regulated by association with multiple regulatory subunits known as cyclins (see Bloom and Cross, 2007, for a recent review). Nutrient availability also modulates the degree of asymmetry of cell division: poor media usually yield large parent cells and very small daughters, whereas in rich media the asymmetry between parent and daughter cells is substantially reduced (reviewed in Porro et al., 2009).

Two quantitative parameters characterize each exponentially growing yeast population: the constant of exponential rate of growth (λ, min^{-1}) and the critical cell size at the entrance into S phase (P_S), (Hartwell and Unger, 1977) (see Alberghina et al., 2009a for a recent review). The value of P_S can be estimated knowing the average protein content (a measure of cell size) and relevant cell cycle parameters of the population under study (Alberghina and Porro, 1993) or it can be directly determined by two-dimensional flow cytometry (Porro et al., 2009). Depending on their genetic set-up, yeast cells can use different carbon and nitrogen sources for their biosynthetic requirements. Average volume, protein content and Ps are nutritionally modulated: higher growth rates and larger P_S are observed in rich media (Alberghina et al., 2004; Hall et al., 1998; Johnston and Singer, 1980; Lord and Wheals, 1980; Vanoni et al., 1983).

In the following we are going to recapitulate how nutrient availability, metabolism and growth control cell cycle progression in budding yeast.

2. Growth controls cycle

The seminal experiment on the relation between growth and cycle in budding yeast was performed in 1977 by Johnston et al. (1977). By inhibiting cell cycle progression (using temperature sensitive cell cycle mutants) or by limiting growth (by inducing nutritional deprivation), they were able to show that mutants blocked at various stages of cell cycle were mostly able to continue to growth as shown by their increase in cell volume, mass and protein content, while inhibition of growth constantly yielded small unbudded cells arrested before the G1/S transition. The exception to this rule is given by early START mutants (such as *cdc25*) that strongly reduce growth, since they behave as nutrient starved cells (Martegani et al., 1984; Pringle and Hartwell, 1981).

On the basis of these findings it was proposed that growth, rather than progression through the DNA division cycle, is usually rate-limiting for cell proliferation, results confirmed later by Hartwell and Unger (1977).

Cell growth during a cell cycle is taken to follow an exponential kinetics. Using a flow cytometric procedure based on staining of cell walls with a fluorochrome-conjugated lectin and cell sorting (Porro and Srienc, 1995), the dynamics of cell protein content increase of a cohort of selected cells born at the same time was determined. Yeast populations growing with different specific growth rates and double-tagged with the procedure described above were analyzed by two-dimensional flow cytometry. The specific growth rate of the daughter cell subpopulation is almost identical to the specific growth rate of the overall population as determined by the increase in cell number (Porro et al., 1995). Furthermore, since the specific growth rate value for the daughter subpopulation and for the overall population is the same in all the tested conditions, it follows that also the parent subpopulation should grow with the same specific growth rate. These data do not exclude that small deviations from the exponential law of individual cells may be detected, for instance by microscopic examination of the cell volume increase of individual cells grown on agar plates (Wheals