Intrinsic, periodic and tunable metabolic dynamics: a scaffold for cellular coherence

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Academic summary

The cell cycle is the periodic alteration between biomass production and segregation. According to the traditional view on cell cycle regulation, waves of cyclins, in strict temporal order, control the cyclin dependent kinase (CDK), which orchestrates the different processes of the cell division cycle. However, there are cues pointing towards cell cycle regulators external to the cyclin/CDK machinery. First, the cell cycle can commence even in the absence of all early cyclins. Second, global transcription and late cell cycle oscillations persist even in the absence of periodic cyclin/CDK activity. Third, a phylogenetic analysis of cell cycle control kinases, indicates that CDKs appeared late in the evolution of eukaryotes, and thus early eukaryotes must have a employed other means to regulate the cell cycle.

The starting point of this thesis was the hypothesis that a metabolic oscillator exists, and acts as a cell cycle regulator. A metabolic oscillator could exert cell cycle control via the oscillating concentration of metabolites, known to interact with the cyclin/CDK machinery and also regulate biomass formation via the epigenetic activation or silencing of growth related genes. In order to test our hypothesis, we first investigated if a metabolic oscillator exists. Using metabolite sensors and cell cycle reporters, combined with microfluidics and epi-fluorescence time-lapse microscopy, we found metabolic oscillations in ATP and NAD(P)H levels in single *Saccharomyces cerevisiae* cells, in the absence of synchronization and intercellular communication. The oscillating metabolism maintains frequency synchrony with the cell cycle across metabolic modes (fermentation, respiration, gluconeogenesis) and growth rates, but also orbits in the absence of cell division, in G1-arrested cells, treated with the mating pheromone alpha factor, cells stochastically skipping cell division on high glucose, or G0 cells occurring under glucose-limited conditions. Based on these findings we concluded that the yeast metabolism is a cell cycle-autonomous oscillator.

The strict synchrony between the oscillating metabolites and the cyclin/CDK machinery in dividing cells, suggests a system of coupled oscillators, consisting of the metabolic oscillator, the early and the late cell cycle. We found metabolism and the cell cycle to exhibit a number of characteristics, representative for systems of coupled oscillators, similarly to what otherwise is found in nature (e.g. neuronal networks or the synchronized discharge of cells in the sinoatrial node). First, we witnessed the proportionality between the compromise and the natural metabolic frequencies. The cell cycle, possibly due to its biosynthetic demands, slows down the metabolic oscillator upon coupling. Second, coupling between metabolism and the cell cycle is achieved only when their frequencies are proximal. When metabolism orbits too slow or too fast, it cannot compromise with the cell cycle to a common frequency and thus cells are unable to divide. We found a clear metabolic frequency threshold robustly separating dividing from non-dividing cells. Third, we found the metabolic oscillator to

robustly gate the phase of the early and the late cell cycle during steady or perturbed growth. The synchrony between the metabolic oscillator and the early cell cycle (biomass formation and DNA replication) persisted even when we halted the late cell cycle, suggesting a direct coupling between metabolism and the early cell cycle.

In this thesis, perturbations served as necessary tools to investigate the interactions between periodic processes, such as the metabolic oscillator and its coupled cell cycle. Nutrient switches were used to perturb the frequency and phase of the metabolic oscillator, and the auxin-inducible degron was used for the conditional and dynamic depletion of cell cycle proteins. Before we could use the auxin-inducible degron system, we had to establish and to thoroughly characterized it on the single cell level. We measured the depletion and recovery dynamics of the targeted proteins for a wide range of auxin concentrations in microfluidics experiments. Although, the auxin-inducible degron exhibits fast protein depletion dynamics (approx. 20 minutes for complete protein depletion), the full recovery of the targeted proteins after removal of the plant hormone auxin requires several generations. This time is crucial for the cells to achieve an equilibrium between protein synthesis and dilution. Here, we also raise awareness on the growth defects caused by the plant hormone auxin, especially when combined with blue light illumination and GFP measurements. With microfluidics becoming largely accessible and the development of novel single cell reporters, we anticipate that our work will facilitate single cell perturbations, exploiting their unprecedented potential in studying dynamic processes.

Investigating the mechanisms responsible for cell-cycle autonomous metabolic oscillations, we studied the role of the cAMP/PKA pathway on the metabolic dynamics, using conditional protein depletion, together with gene knock-outs. . We found Rim15, the activator of stress response and inhibition target of cAMP/PKA signaling, to operate as a metabolic attenuator, dampening the amplitude of the metabolic oscillations, in the absence of cAMP signaling. Such a metabolic attenuation is necessary for the robust cell cycle arrest upon the addition of the mating pheromone alpha factor. Our results indicate that the cAMP/PKA pathway, known to be activated by flux-dependent metabolite signals, provides positive feedback to the metabolic dynamics, via the inhibition of stress response and possibly also via the liquidation of carbon storage.

Overall, this work establishes yeast metabolism as a cell-cycle autonomous oscillator and provides evidence that cell cycle control is a higher order function emerging from the collective synchrony between coupled oscillators, including the metabolic and the cyclin/CDK oscillator. Because central metabolic pathways are conserved across all taxa of life, the metabolic oscillator may constitute a primitive cell cycle regulator, also present in early eukaryotes prior to the appearance of the cyclin/CDK machinery. Additional control, such as the early cyclins and the onset of CDK activity or nutrient signaling, was possibly engrafted on the metabolic oscillator, entraining the metabolic

dynamics for the robust separation of the cell cycle phases, or the robust cell cycle initiation when nutrients are abundant. If a similar metabolic oscillator also exists in mammalian cells, then it may provide novel therapeutic targets against proliferative disorders.