CELL-SIZE REGULATION MECHANISMS IN G1

Kim van den Handel, 6663524
03-02-2022
Cancer, Stem Cells and Developmental Biology – Utrecht University

ABSTRACT

Cell size regulation is thought to be regulated by either the dilution of cell cycle inhibitors or the accumulation of activators. Dilution of cell cycle inhibitors occurs via similar molecular mechanisms across organisms as the proteins involved are conserved cell cycle regulators, such as Whi5 in yeast, Rb in animal cells and KRP4 in plants. These cell cycle proteins are thought to be the size sensor of the cell, maintaining a uniform cell size in the population for optimal cell fitness. On the other hand, accumulation of start activators has mostly been investigated in yeast, involving the accumulation of Cln3 and Swi4 (SBF factor) START regulators. In this review I explore the molecular mechanisms involved in cell size regulation in yeast, animal cells and plants and discuss the variations between different studies. I also present a different size sensor mechanism that is reported in mammalian cells. A crosstalk between upstream MAPK p38 and CDK4 allows for cell cycle progression only until a certain size threshold has been reached that is suitable for the environment.

LAYMEN'S SUMMARY

A cell's size is important for optimal fitness of the cell and as cell size is uniform within a specific cell type, it is thought to be regulated by internal factors that govern cell division. Cell division is orchestrated by sequential phases in a cycle. The first phase is called G1 which is ended by a checkpoint during which various factors are checked in order to continue with the other phases of the cell cycle. One of these factors is a cell's size. A cell must have grown enough to reach a certain threshold to progress to the second phase. The checkpoint checks a cell's size using proteins that 'report' the size of a cell to the cell division machinery. This can be a protein that would block the progression of the cell cycle, and is diluted as a cell grows. When a cell reaches a certain size (threshold), it is allowed to continue in the cell cycle. Similar, a the protein that reports the size of a cell could be an activating protein which increases in abundance simultaneously with cell growth. When reaching the size threshold, the cell cycle progresses. The behaviour of proteins that report the size of a cell is partly determined by the environment of the cell. As coordination of cell size regulation involves both internal and external factors, it is interesting to investigate what determines a specific cell size and what molecular mechanisms are involved to constrain cell size uniformity and maintain optimal cellular function.

Supervision: Suzanne Lens
Secondary review: Martijn Gloerich

CONTENT

INTRODUCTION

In a general context, the specific size of a cell is the outcome of numerous and diverse processes. Originally, It was thought that growth and proliferation were separate processes of the animal cell due to specific stimulation of either process by insulin-like growth factor (IGF) or epidermal/glial growth factor (EGF/GGF respectively) (I. J. Conlon et al., 2001; Hall et al., 2004; Zetterberg et al., 1984; Zetterberg & Larsson, 1991). This did not explain, however, how cell size variances are constrained to maintain size uniformity that is typically found in tissues. Although extracellular signals can influence the mean cell size of a population, individual cells will deviate from that mean. This suggests that cells reduce size variation in a population using intracellular processes that differentially affect cells of different sizes, even though they share the same environment. From this thought, the idea of a size-sensor or sizer within the cell arose (Fig. 1; Box 1).

Early microscopy experiments had shown that early S phase cells were less variable in size than those in early G1, identifying the G1/S cell cycle transition as an important cell size regulation point (Killander & Zetterberg, 1965). This size sensor limits size variability by progressing through the cell cycle in a size dependent manner. The concept being that small cells need more time to grow (longer G1) compared to oversized cells (Dolznig et al., 2004; Killander & Zetterberg, 1965) (Fig. 1). A size threshold has been researched for budding yeast (Johnston et al., 1977), but has gained more attention for animal cell research since (M. Ginzberg et al., 2018). Blocking cell cycle progression resulted in larger cells (Fingar et al., 2002), while blocking cell growth prolonged the cell's G1 phase (Tan et al., 2021). In addition, small cells may have an increased growth rate compared to larger cells at birth. Together, this indicates a compensatory mechanism that aims to balance growth and cell cycle progression in mammalian systems. In order for this mechanism to operate, cells must be able to report their size to processes that orchestrate the progression of the cell cycle (Fig. 1). Recently, it became evident that this signal could be for example the dilution of a regulatory protein or the accumulation of a transcription factor.

The regulation of a cell's size has received more attention in particular, as cell size can affect various biological functions of the cell. Aside from the obvious physiological influences of cell size on cell functionality such as surface to volume ratio and membrane receptor availability, differences in gene expression and metabolic activity between large and small cells have been reported for mammalian adipocytes (Farnier et al., 2002, 2003), hepatocytes (Miettinen et al., 2014) and erythrocytes (Maciak et al., 2014). Moreover, cell size often changes when a cell differentiates, indicating the connection between cell size and function. Lymphocytes for example, rapidly grow larger in response to Toll-Like Receptor stimulation (a signal for differentiation), suggesting that the specialized function of the matured lymphocyte requires a certain size (Abbas et al., 2016). These findings collectively strengthen the idea that cell size regulation is involved in a more complex regulatory network than mere surface and volume availability for the processing of signals.

Notably, modulation of organ growth through cell growth or proliferation is often context-dependent. Pancreatic beta cell size can for example increase during pregnancy in response to the increased demand for insulin, but will proliferate in response to increased beta cell death (Dhawan et al., 2007). Also, epithelial kidney cells modulate their size in response to the mechanical shear on primary cilium caused by the rate of fluid flow (Boehlke et al., 2010). However, as one cell can have multiple roles, it is difficult to determine whether the size of a cell is cause or consequence to its function.

Despite the fact that an organ can be composed of different cell types varying in size, cells from the same cell type generally share a uniform (target) cell size. Size sensing in this context is thought to be required to maintain optimal cellular fitness. Transplantation assays with mouse Haematopoietic Stem Cells (HSC) showed that optimal cellular fitness was reached for medium sized HSCs, suggesting a target cell size for optimal functioning of the cell (Lengefeld et al., 2021). Furthermore, relevance for cell size maintenance is also demonstrated by diseases that may emanate from disturbances in cell size regulation. Increased adipocyte size, instead of total body fat, is associated with insulin resistance in obesity and is a risk factor for development of type II diabetes (Guilherme et al., 2008). Additionally, aging is associated to decreased cellular fitness due to excessive cell enlargement and a decreased DNA:cytoplasm ratio (Neurohr et al., 2019). A notable example for this trend also includes cancer cells. Cancer cells demonstrate that loss of cell size maintenance (size heterogeneity), results in loss of their function or order, which they originally possessed from the organ they arose from. As size heterogeneity can be a sign of neoplastic growth, it indicates that the size and function of a cell are highly connected, highlighting a biological argument for cells to maintain a specific target size.

A more specific context for cell size regulation is being investigated on protein level. Regulatory networks that influence cell cycle checkpoint transitions and cell growth involve either the dilution of inhibitory factors or accumulation of stimulatory factors (Dorsey et al., 2018; Schmoller et al., 2015). Dilution of cell cycle inhibitors like Whi5 in yeast or Rb in animals have been described as inhibitor dilution model for cell size regulation (Barber et al., 2020; Constanzo et al., 2004; de Bruin et al., 2004; Schmoller et al., 2015; Zatulovskiy et al., 2020). On the other hand, accumulation of cell cycle related transcription factors relative to their binding sites such as SBF in yeast and E2F in animals and plants, are attributed to the titration model (de Bruin et al., 2004; Dorsey et al., 2018; Heldt et al., 2018). Typically, the dilution of inhibitory factors, and the accumulation of stimulatory factors fit within the mathematical sizer model criteria, where a size-senor is thought to link cell growth and proliferation (Box 1). Interestingly, recent studies in animal cells have suggested upstream signal transduction pathways that even better define this coordination of cell size and cell cycle regulation (Liu et al., 2018; Tan et al., 2021).

Despite the simplicity of size as a phenotype, its study is inherently challenging, as the specific size of a cell is an outcome of numerous and diverse processes. Measuring a cell's individual size is thought to be particularly challenging and studies use different techniques to do so. Moreover, studies investigating cell size regulation are mostly done using proliferating cell cultures, whereas most of the cells in our body do not sustain proliferation. This review will focus on the regulatory mechanisms on protein level that determine the balance between cell growth and timing of division as a result of G1/S checkpoint regulations, to maintain a uniform target cell size in budding yeast, animal cells and plant cells. First, an overview of the current perspective on cell size regulation mechanisms will be presented, after which a discussion will follow about the differences in study approaches that led to the conclusions of the discussed literature.

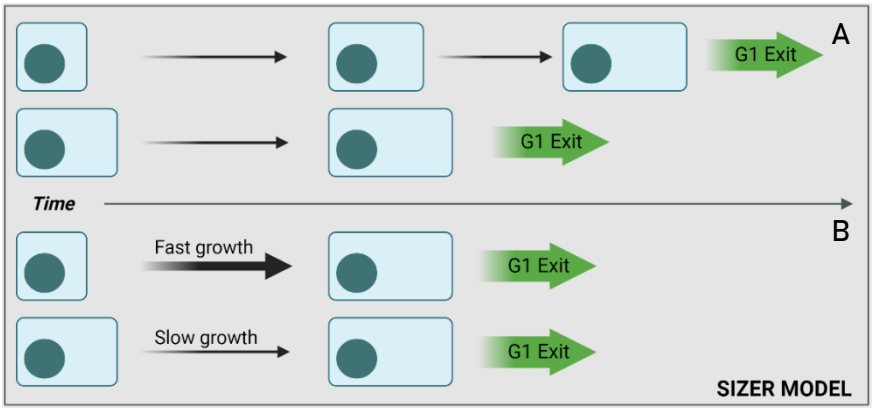
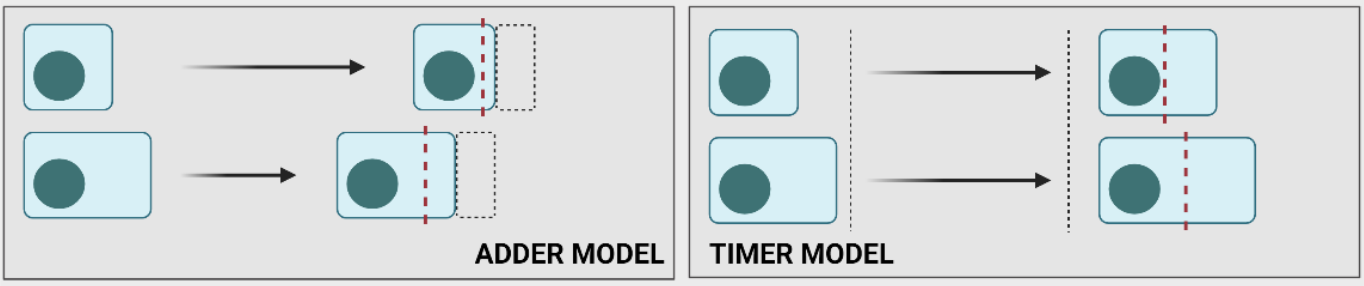


Fig. 1: Strategies for size control. Cells can either compensate for their small size with **(A)** a longer G1 or **(B)** an increased growth rate. Adapted from (M. B. Ginzberg et al., 2015), Created with Biorender.com.

BOX 1: MATHEMATICAL SIZE MODELS

Mathematical models have described cell size maintenance by either "sizer"(Facchetti et al., 2017; Garmendia-Torres et al., 2018; Heldt et al., 2018), "adder"(Cadart et al., n.d.; Taheri-Araghi et al., 2015) or "timer"(Jones et al., 2017) models. The sizer model describes the dynamics of cell growth and G1 length compensation to obtain a uniform cell size. The regulation is based on a target size that grands progression to the S-phase of the cell cycle (Fig. 1). Adder models, on the other hand, propose a size regulation in which the growth of every cell in the population is constant, such that the division is triggered after fixed growth increment. Large cells reach the fixed growth increment faster. Variation in cell size is diminished over several cell cycles (not shown in figure below) if cells divide symmetrically, until their increment is equal to their birth size. Lastly, the timer model relies on a linear growth (steady-state) in a population of cells to obtain cell size uniformity. In this model, divisions are set after a fixed time period and large and small cells divide synchronously. However, cell size simulations of plant cell sizes suggest that the timer model is not sufficient to constrain the distribution of cell sizes in the Shoot Apical Meristem and that differences in cell size are rather amplified (Jones et al., 2017). As not all experimental data fits these three models, combinations of the three are also being proposed such as "imperfect sizers" and "dynamic-adders"(Heldt et al., 2018; Tanaka et al., 2021).



Adder and Timer models. Divisions are schematically represented by red dotted lines. Black dotted lines indicate specifics of the models, either a fixed increment (adder) or a fixed growth period (timer). Adapted from (Jones et al., 2017), Created with Biorender.com.

G1 TO S-PHASE TRANSITION OF THE CELL CYCLE

The cell cycle is a series of events divided in four phases, that allow a cell to grow and multiply. Chromosome duplication and genetic distribution to daughter nuclei is orchestrated during the S and M phases of the cell, at the end of which the cell divides into two during a process called cytokinesis. The S- and M-phases are separated by GAP phases – a G1-phase between M- (mitosis) and S-phase and a G2-phase between S and M (Fig. 2). These GAP phases allow the cell to grow and assess its internal and external environment to ensure that conditions are suitable for cell division. The assessment of the environment is facilitated by the cell cycle control system that governs cell cycle progression at three specific checkpoints (yellow, Fig. 2). The first one is the G1/S commitment point called START (in yeast cells) or restriction point (in mammalian cells), during which (overall) gene expression is increased. The second is the G2/M transition and third is during the metaphase to anaphase transition in mitosis. Central to the regulation of these checkpoints are the cyclin-dependent kinases (CDKs). These proteins form active complexes with specific cyclins, the type of which depends on the cell cycle phase. As the name predicts, cyclins undergo cycles of synthesis and degradation with every cell cycle (Fig. 3). In contrast, CDKs levels are practically constant. It is because of the 'cycling' concentrations of cyclins that CDK-Cyclin activities can be orchestrated at specific stages of the cell cycle. Apart from an activating function, cyclin proteins are able to direct its CDK partner to specific target proteins. This allows the same complex to induce different effects at different times in the cell cycle. Here, only G1/S cyclins and CDKs are discussed, as this review focuses on the G1/S regulation of the cell cycle in relation to cell size (Alberts et al., 2008).

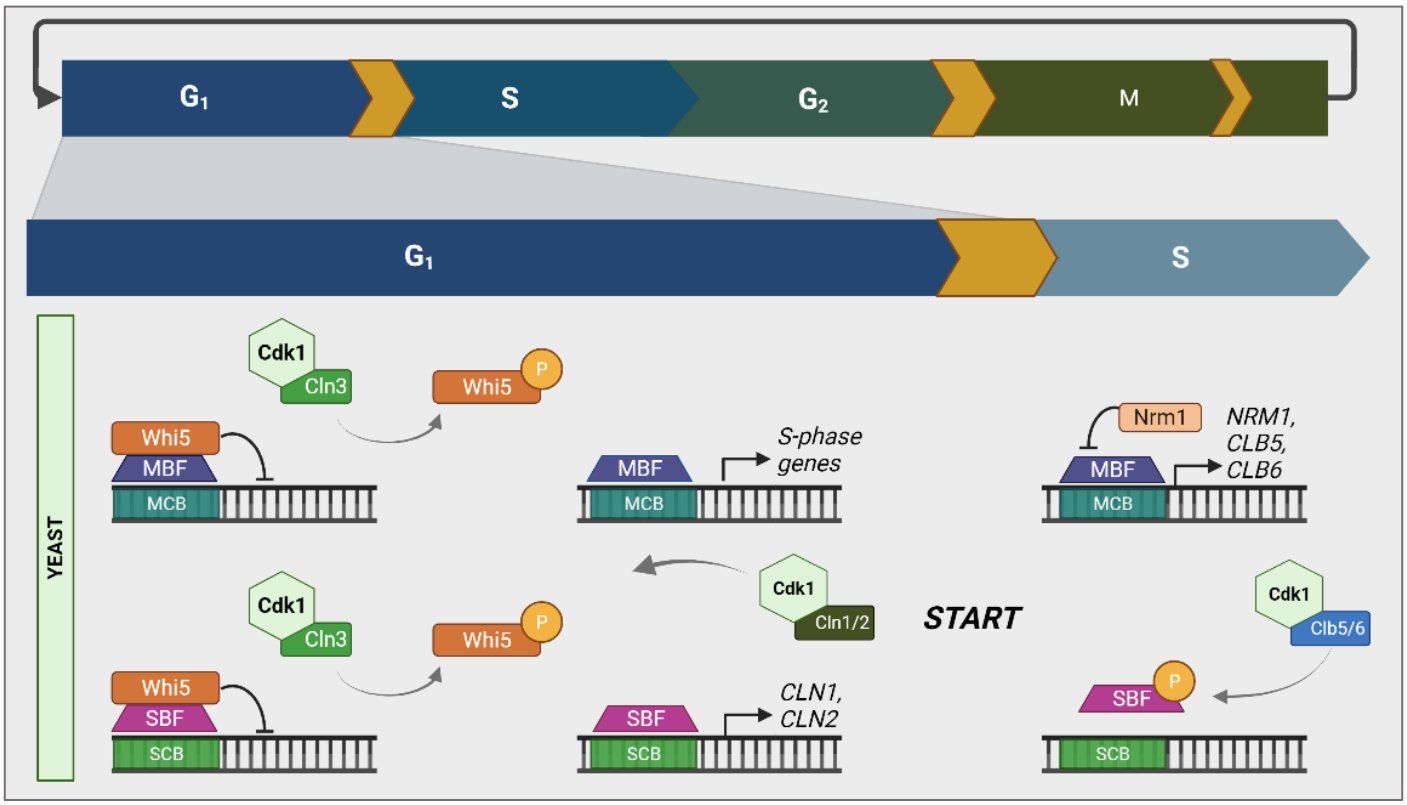


Fig. 2: G1/S regulation in yeast. G1 cell cycle regulation involving G1/S inhibitor Whi5, phosphorylated by Cdk1-Cln3. Subsequent transcription of Cln1/2 by SBF factors triggers the activation for irreversible G1 progression: START. Created with Biorender.com

As the proteins regulating cell cycle control are so well conserved over the course of evolution, all eukaryotes use similar machinery to regulate cell cycle events. However, there are slight differences. In budding yeast, a single Cdk binds all classes of cyclins (Cln), the active complex in G1 being Cln3 and Cdk1 (also known as Cdc28). In early G1, the Cln3-Cdk1 complex is bound to the ER in the cytoplasm. As G1 progresses, Cln3-Cdk1 is transferred to the nucleus (chaperoned by Ydj1), where it phosphorylates transcriptional repressor Whi5 (Fig. 2) (Ferrezuelo et al., 2012; Vergé et al., 2007). As a result of this phosphorylation, Whi5 is released from SBF transcription factors (TFs), which induces nuclear export of Whi5 and G1/S transcriptional activation (START) . This activation induces a positive feedback loop with Cln1 and 2 that, in complex with Cdk1 further inhibit Whi5, resulting in the irreversible activation of START (also known as the G1-S regulon) (Skotheim et al., 2008). START transcription is induced by SBF (SCB-Binding Factor) TFs and is repressed by MBF (MCB-Binding Factor) TFs in early S-phase. SBF transcription factors consist of subunits Swi4 and Swi6, whereas MBF transcription factors are comprised of Mbp1 and the shared subunit Swi6.

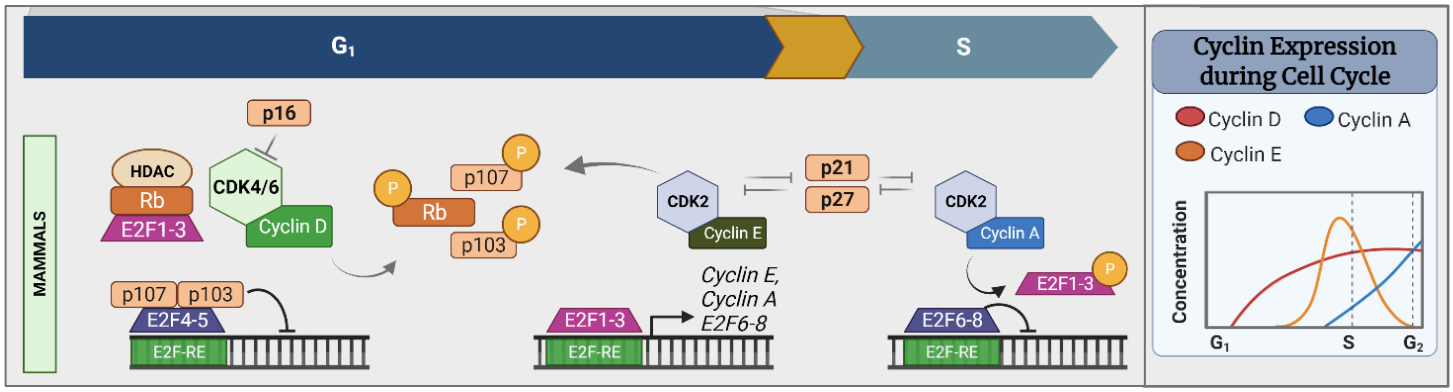


Fig. 3: G1/S cell cycle regulation in mammalian cells. G1 cell cycle regulation involving G1/S inhibitor Rb, phosphorylated by Cdk4/6-Cyclin D. Subsequent transcription of Cyclin E is initiated by E2F1-3 and triggers the restriction point. Created with Biorender.com.

In mammalian cells, the activation of G1/S is induced by E2F (adenovirus E2 promotor binding factor) TFs. In early G1, these E2F factors are repressed by either retinoblastoma proteins (Rb) or p103 and p107, collectively known as pocket proteins (Fig. 3) (Bertoli et al., 2013). Upon growth stimulation by for example mTOR activation, repression of E2F1-3 (activators) is released by CDK4-Cyclin D (orthologous to yeast Cdk1-Cln3) dependent phosphorylation of Rb, the functional orthologue of yeast's Whi5. Phosphorylation of p103 and p107 on the other hand, facilitates the nuclear export of E2F 4 and 5, releasing the occupation and repression of the G1 promotor. Subsequently, a positive feedback loop is induced by the CDK2-Cylin E complex. As the cell progresses into S phase, this amplification is counteracted by Kinase Inhibitor Proteins (KIP) p21 and p27, repressing G1 Cdk activity. This repression facilitates a switch-like entry into S-phase and maintains G1/S irreversibility, which is accompanied by G1 promotor repression by E2F6-8 (Alexis Barr et al., 2016).

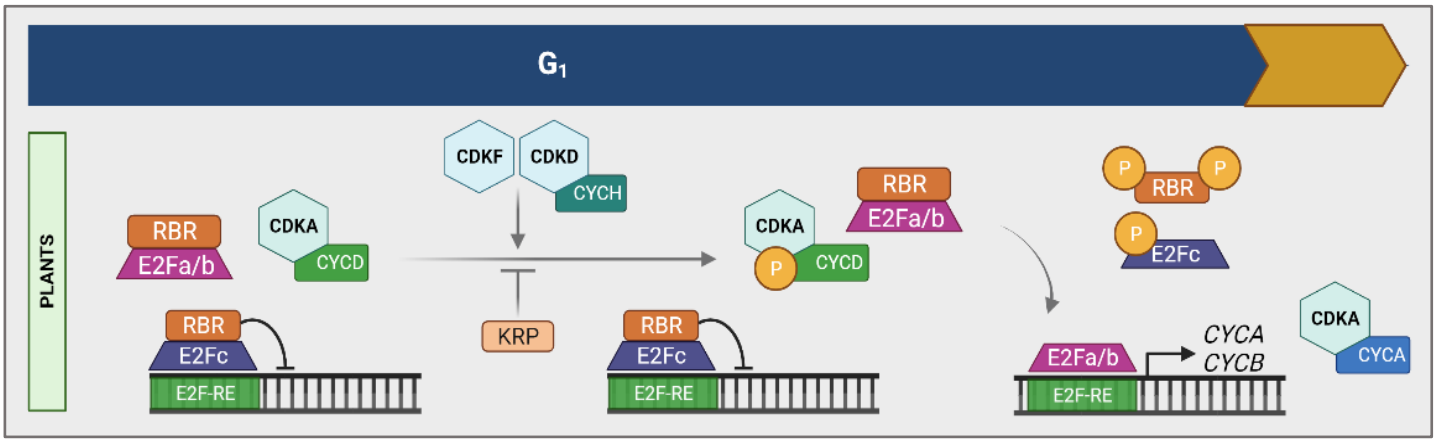


Fig. 4: G1/S regulation in plants. G1 cell cycle regulation involving G1/S inhibitor RBR, phosphorylated by CdkA-Cyclin D. Subsequent transcription of ClnA/B by E2F factors triggers the activation for irreversible G1 progression. Created with Biorender.com.

Similar to mammalian cells, plant cells activate their G1/S transition using E2F factors (E2Fa/b), which are retained by the Rb-related protein RBR in early G1 (Shen, 2002). In the presence of growth stimulatory factors, CDKA forms an inactive complex with cyclin D (CYCD) (Fig. 4). This complex is activated by cyclin activating kinases (CAKs) CDKF and CDKD-CYCH. Functionally orthologous to mammalian CDK4/6-Cylin D, activated CDKA-CYCD facilitates phosphorylation of RBR in plants (Inzé & de Veylder, 2006). Subsequently, the expression of G1/S-phase genes will be activated by nuclearization of E2Fa/b, and degradation of E2Fc by the SCF E3-ubiquitin ligase complex. Moreover, similar to the p21 and p27 proteins, KIP Related Protein (KRP) can inhibit the activated CDKA-CYCD complex in response to unfavourable growth signals.

Regulation of these GAP phases is important to prevent cells from getting smaller after every cell division. In mammalian cells, blocking cell cycle progression with p16 resulted in larger cells (Fingar et al., 2002), while blocking cell growth by inhibition of mTOR prolonged the cell's G1 phase (Liu et al., 2018; Tan et al., 2021). Adaptation of G1/S allows the cell to compensate when its size deviates from the target cell size in a tissue. This compensatory mechanism that balances growth and G1 duration was first discovered budding yeast (Johnston et al., 1977).

CELL SIZE CONTROL IN BUDDING YEAST

The balance between cell size and the duration of the cell cycle in yeast has served as a uniform model to uncover the underpinning mechanism. The most recent debate in yeast focuses on the contribution of the dilution of cell cycle repressor Whi5 or the titration of SBF/MBF transcription factors to their corresponding binding sites. These mechanisms are also referred to as the inhibitor dilution model and the activator accumulation model respectively. A larger cell in G1 would either have a diluted repressor or have grown enough (at the end of G1) to have accumulated sufficient START activators (Son et al., 2012).

INHIBITOR DILUTION MODEL: WHI5

Support for the dilution model is mostly focused on the interplay between cell cycle repressor Whi5 and its regulator Cln3 (Constanzo et al., 2004; de Bruin et al., 2004; Schmoller et al., 2015). The role of Whi5 inactivation in transcriptional amplification of around 200 genes during START marks the importance of this protein in G1 cell cycle regulation (Fig.2) (Hartwell et al., 1970; Skotheim et al., 2008). Although the Cln3-Cdk1 dependent phosphorylation of Whi5 during the activation of START has been known for over almost two decades, elucidating the specific balance and contribution of these proteins in the regulation of cell size during cell division has been challenging.

In 2006, it was discovered that Whi5 is only being transcribed during S/G2/M phases of the cell cycle, and not in G1 (Pramila et al., 2006; Schmoller et al., 2015). After cell division, this causes higher concentrations of Whi5 in small daughter cells than in large daughter cells. Because the concentration of Whi5 is set before the next division, this regulatory protein functions as a memory that is inherited to maintain cell size homeostasis in the population (Constanzo et al., 2004; Schmoller et al., 2015). Small daughter cells harbouring high Whi5 concentrations seem to extend their pre-START G1 cell cycle phase to dilute Whi5 transcriptional inhibition until a target size has been reached (Fig. 1). Translation of Cln3 then modulates the rate at which cells can pass START, its concentration remaining practically constant as its syntheses is thought to increase proportionally to cell size. Reaching a target cell size balances the Whi5:Cln3 ratio such, that Whi5 is being released from SBF transcription factors (Fig. 2) (Heldt et al., 2018; Schmoller et al., 2015). This data suggests that Whi5 dilution would be the cell size regulatory mechanism in budding yeast.

A small refinement to this model is proposed by experiments that uncoupled Whi5 expression from its endogenous promotor. Barber et al. (2020) conclude that linear scaling of Whi5, compared to endogenous sub-scaling, does not result in an increase in cell size variation, indicating that the Whi5 dilution model is not the dominant mechanism constraining cell size distribution in wild-type budding yeast. Nevertheless, they showed that increasing Whi5 resulted in a larger average cell size, and that the negative correlation between G1 duration and cell size at birth was maintained (Fig. 1).

Interestingly, a most recent discovery shows that the scaling of cell cycle related proteins during G1 is not identical for every protein (Swaffer et al., 2021). Rather, while most proteins scale with cell size, other proteins sub scale or even super-scale (Fig. 5). For example, histone synthesis is matched to genome content rather than cell size, resulting in histone concentrations in the daughter cell that are less compared to what would be expected proportionally to cell size (sub-scaled). In particular, Whi5 was shown to sub-scale as Whi5 synthesis is independent of cell size and occurs mostly during S/G2/M in a fixed time window, and in an environment dependent manner (Pramila et al., 2006; Schmoller et al., 2015). This sub-scaling is further facilitated by chromatin-binding to partition similar protein amounts to each new-born cell regardless of daughter cell size. Disrupting both Whi5 synthesis and chromatin-based partitioning weakened G1 size control, consistent with the original dilution model (Schmoller et al., 2015; Skotheim et al., 2008). In summary, the inhibitor dilution model in budding yeast explains the inverse correlation between G1 length and cell size at birth by sub scaling of the Whi5 cell cycle repressor (Fig. 5). As Whi5 is only synthesized during S-M phases, it is thought that cell-size-relevant information about the environment is transferred to daughter cells via the inherited partitioning of Whi5 by means of chromatin binding. This way, daughter cells can adjust their G1 length such, that the Cln3-Cdk1 dependent phosphorylation of Whi5 effectively results in the release of Whi5 from START transcription factors (Fig. 1 and 2).

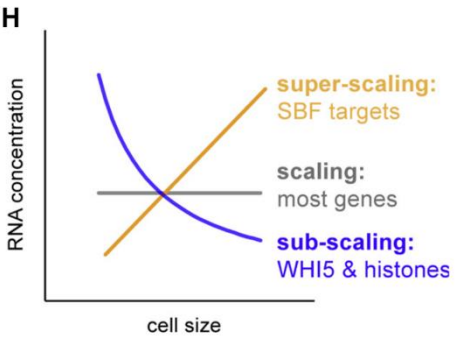


FIGURE 5: ADOPTED FROM: (SWAFFER ET AL., 2021) SCHEMATIC REPRESENTATION OF THE DIFFERENT SCALING OF PROTEINS.

ACCUMULATION OF START ACTIVATORS: Cdk1-CLN3 DYNAMICS

On the other hand, there is a rising interest in the dynamics of START activators. The basic mechanism of this relies on the availability of transcription factor binding sites (SCB and MCB elements) that activate ~200 genes for the commitment to START and the number of activators (SBF transcription factors) that occupy these elements. Because SBF factors are released from their inhibition by Cln3-Cdk1 dependent phosphorylation of Whi5, the accumulation of Cln3 is considered an activator of START, dictating the activity of the kinase in G1 (Skotheim et al., 2008).

One of the first indications that linked the titration of Cln3 to cell size regulation was described by Wang et al., (2009). They showed that the START/*CLN2* promotor, is able to bind a (Cdk1-)Cln3-SBF complex on SCB elements leading to the release of Whi5 from SBF and activation of transcription (Fig. 2, 6). As Cln3 is the dynamic and activating part within this complex, Wang et al. investigated how these Cln3 levels relate to the SBF binding sites in the promotor of START. ChIP and transformation assays let them suggest that, Cln3 is only 'sub-stoichiometric' with respect to the functional SBF binding sites. In other words, there are more SBF binding sites than there are Cln3 molecules in the cell (in a 1:4 ratio). In addition, the MBF and SBF dimer copy numbers are not even 1/3 of the total G1/S promotor sites in small cells, whereas in large cells they outnumbered the total number of G1/S promotor sites.

Genetically increasing the number of SCB elements in the genome resulted in larger cells, an effect that was compensated by extra introduction of *CLN3* in the genome to increase the Cln3 concentration independent of size. This indicated that although (Cdk1-)Cln3 molecules do not fully occupy the SBF binding sites in G1, the increase in Cln3 during G1 growth causes an increase in fractional occupancy of the binding sites until a threshold has been reached at a corresponding target cell size. This threshold causes sufficient Cdk1-Cln3 dependent activation to initiate Cln2 expression (amplification) and other SBF targets to induce START. As both Cln3 and SBF subunits are not able to fully occupy promotor sites, active SBF and MBF are stoichiometrically limiting in small new-born daughter cells. This effect is diminished as the cells grow/progress through in G1 (Black et al., 2020). As the amount of Cln3 is cell size and growth rate dependent, it suggested that the titration of SBF factors is necessary to activate START (Schmoller et al., 2015; Wang et al., 2009).

Titration of Cln3 mediated activation of SBF is linked to a cell's size due to the growth dependent proportional increase of Cln3 (Schmoller et al., 2015; Wang et al., 2009). However, the increase in Cln3 during growth in G1 might not be linear as was concluded from experiments with stabilized Cln3 (Schmoller et al., 2015). Recently, single cell time-lapse fluorescent microscopy and bicistronic experiments shed a new light on the dynamics of Cln3 during cell cycle progression. A viral based bicistronic construct allows for the expression of two genes within the same mRNA construct. In this case, Cln3 was coupled to GPF, such that Cln3 and GFP are expressed together and separated upon translation. Using this technique allowed Litsois and collegues to directly measure Cln3 translation as a function of GFP intensity, without having to artificially stabilize the protein. The experiments showed that Cln3 is being produced in pulses throughout the cell cycle, following the intrinsic metabolic activity of the cell (Litsios et al., 2019). Interestingly, the increase in Cln3 in G1 is proportionally larger than the increase in cell size, which facilitates a Cln3 magnification that stimulates START. This differential scaling has been shown to be universal for mother and daughter cells and across different nutrient conditions.

ACCUMULATION OF START ACTIVATORS: SWI4

Although the pulse-like production of Cln3 may not be nutrient dependent, nutrient content positively correlates with cell size. High nutrient conditions generally result in larger cells. Possibly, the correlation between nutrient availability and size regulation can be explained by a study that used scanning number and brightness microscopy (sN&B) to measure absolute concentrations of GFP fusions. Dorsey and colleagues reported that the threshold for START activation is set by a nutrient-dependent occupancy of G1/S promotors by SBF subunits. According to this study, this is facilitated by a size dependent increase of SBF subunit Swi4. Consistent with previous results (Litsios et al., 2019), Whi5 amounts did not change, resulting in dilution of Whi5. As un-phosphorylated Whi5 binds SBF at promotor sites, the Whi5:Swi4 ratio and its functional inhibition decreases with increasing cell size (Fig. 6). Poor nutrient conditions resulted in relatively higher Swi4 concentrations, suggesting that cells in these conditions harbour a Whi5:Swi4 ratio that is even smaller. This data indicates that SBF transcription triggers START at a smaller target size in a low-nutrient environment. If nutrient conditions affect Swi4 concentrations and Whi5 inherently transfers environmental information to daughter cells, nutrient availability influences the G1/S target cell size.

In contrast to Litsois and Dorsey et al., Qu and colleagues report that the peak levels of Whi5 are depending on nutrient content (Qu et al., 2019). Time-lapse fluorescent microscopy experiments showed that the maximum Whi5 nuclear concentrations increased with decreasing nutrient content. As Whi5 synthesis rate remained stable across conditions, a prolonged G1 phase was observed with increasing nuclear Whi5 concentrations. This finding is again consistent with the compensation strategy of cells (Fig. 1A) and suggests that an increase in Whi5 may increase the Whi5:Swi4 ratio sufficient to prolong G1 in poor nutrient conditions. In contrast, Skoheim and colleagues (2015) found that Swi4 remained constant throughout G1, when daughter cells are grown in a very low-nutrient environment like ethanol. This last finding marks the complex comparison between different studies on cell size regulation as the experimental setup is highly variable and the choice for a specific nutrient composition might induce bias to the results.

Overall, yeast regulate their cell size by equal partitioning of cell cycle inhibitor Whi5. The concentration of Whi5 allows small cells to compensate for their deviating cell size by prolonging their G1 phase, to grow to the correct (nutrient dependent) target size. While cells are growing in G1, Cln3 G1/S activator increases relatively to the Whi5 inhibitor, titrating its concentration with respect to activating SBF transcription factors. This fractional occupation increases, until a threshold has been reached and G1/S transition is activated.

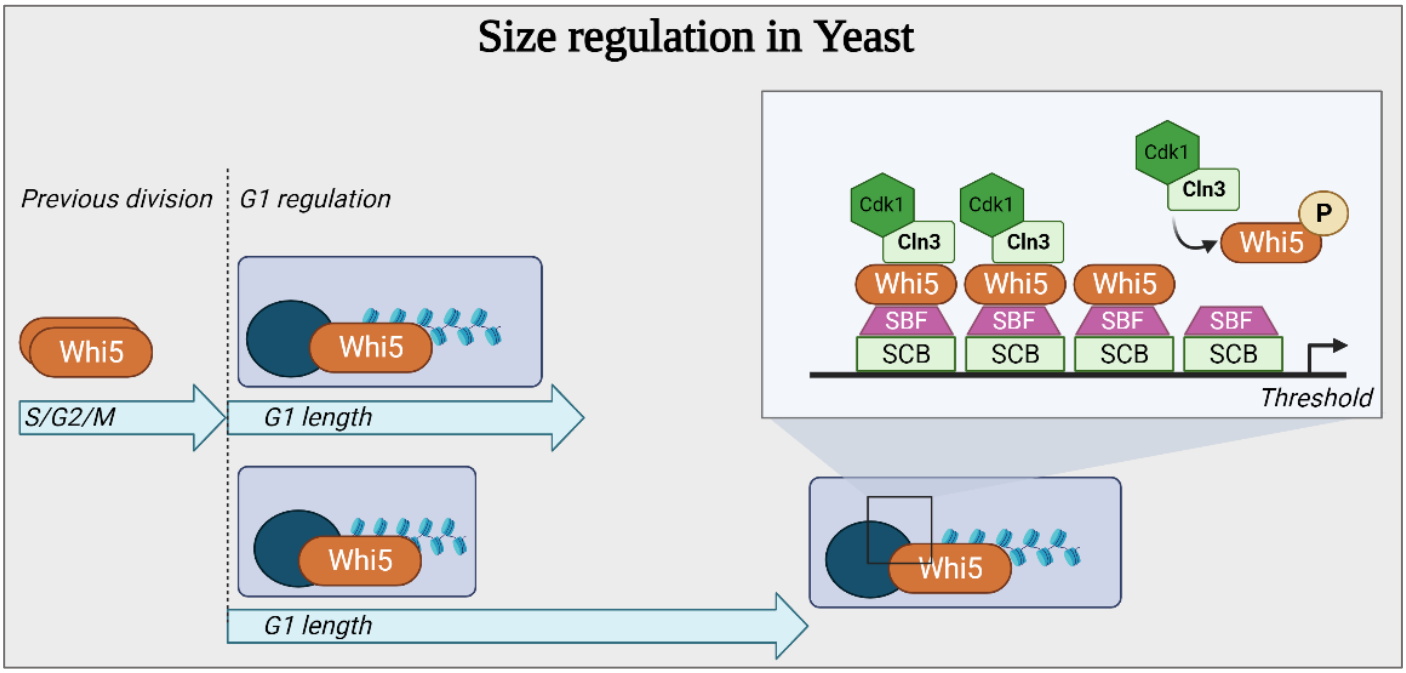


FIGURE 6: SCHEMATIC REPRESENTATION OF G1 REGULATION IN YEAST. CREATED WITH BIORENDER.COM.

CELL SIZE CONTROL IN MAMMALIAN CELLS

As the cell cycle is an evolutionary conserved mechanism, many mammalian cell cycle proteins are analogous to the ones found in yeast. The Rb protein in mammalian cells restrains E2F transcription factors to inhibit the G1/S transition until it is phosphorylated by the CDK4/6-Cyclin D, much like Whi5 retains SBF and is phosphorylated by Cdk1-Cln3 in yeast (Fig. 2 and 3). Consistent with yeast data, the compensatory size regulation strategy affecting G1 length and cellular growth rate is used to correct cells in the population that have failed to fall within the appropriate size range (Fig. 1) (Son et al., 2012).

INHIBITOR DILUTION MODEL: RB

Recent work in mammalian cells shows a striking similarity with the dynamics of Whi5 inhibitor in yeast. Through each cell cycle, different-sized cells produce similar amounts of Rb and equally partition during division, such that new-born cells all inherit a similar amount of Rb. This inheritance resembles the partitioning of Rb analogue Whi5 (Schmoller et al., 2015; Zatulovskiy et al., 2020). However, despite present knowledge about Rb association with chromosomes during mitosis, It is not specifically investigated whether partitioning of Rb is facilitated by chromatin binding.

A more specific analysis on the contribution of the Rb/CDK4-CyclinD axis in size regulation is presented by M. Ginzberg et al. (2018). Compound screenings targeting specific proteins in the cell cycle regulatory network resulted in coordinated changes of both cell cycle length and growth rate, such that cell size remained relatively unchanged. Uniquely, treatment with CDK4/6 inhibitor Palbociclib increased cell cycle length and an unusual increase in cell size, without triggering a compensatory effect on growth rate (like in Fig. 1B). Interestingly, size compensation in Rb-inactive cells was identical to Rb active cells, indicating that size compensation in mammalian cells is independent of Rb activity (M. Ginzberg et al., 2018). Although the influence of CDK4-CyclinD on cell size implies and important role for the Rb-cyclin D axis in size regulation, the pathway may not be responsible for the coordination of growth rate and cell cycle length to maintain cell size uniformity. Moreover, although yeast Whi5 and mammalian Rb are both cell cycle inhibitors, they share no sequence similarity and have a different evolutionary origin, suggesting that the role of Rb in cell size regulation may not be identical to Whi5 (Medina et al., 2016). So what could be the link between CDK4-Cyclin D and cell size regulation?

MAPK SIGNALLING IN CELL SIZE CONTROL

Although the dilution model has been investigated for animal cell size regulation, the accumulation model is not very prominent in literature. Instead, specifically the p38-MAPK signalling pathway is being linked to cell size coordination. In general, Mitogen-Activated-Protein-Kinase (MAPK) pathways are widely studied in mammalian cells and are mostly known for their response to numerous signals including stress, growth factors, cytokines and others. MAPK pathways receive a particular signal via a G-protein coupled transmembrane receptor which transfers the signal to the first kinase of the pathway: a MAPK kinase kinase (MAPKKK) (Zhang et al., 2002). Subsequently, the cascade involves signal transduction to MAPKK and MAPK's that will activate gene transcription in the nucleus. As MAPK pathways are responsive to many stimulating signals, they facilitate signal transduction to induce various processes such as proliferation, differentiation, development and apoptosis. Within this large signalling network, there are three major pathways to be distinguished; ERK1/2, p38 and JNK/SAPK. Of these three, p38-MAPK is known to regulate the G1/S checkpoint by both transcriptional inhibition and stimulating degradation of cyclin D (Fig. 7), but its specific role in size regulation was unknown until recently (Martínez-Limón et al., 2020; Zhang et al., 2002).

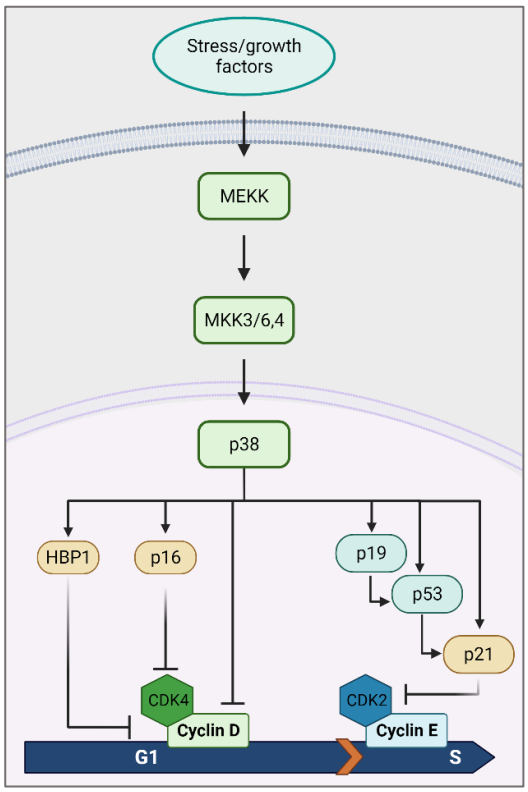


FIGURE 7: P38 MAPK PATHWAY IN G1/S REGULATION (MARTÍNEZ-LIMÓN ET AL., 2020; ZHANG ET AL., 2002), CREATED WITH BIORENDER.COM.

RNAi (Björklund et al., 2006) and small molecule screenings (Liu et al., 2018) identified the p38-MAPK to be involved in the regulation of cell size and G1 length. The small molecule screening first identified on- and off-axis targets, which categorized proteins and their respective pathways into the two categories based on their effect on G1 length and cell size. On-axis targets affect cell size and G1 length, but maintained coordination between the two (Fig. 1). Whereas off-axis targets disproportionally affect cell size and G1 length, resulting in loss of coordination. Targets that scored highest in the off-axis category, were thought to perturb the coordination of cell size and G1 length most. Interestingly, the mTOR pathway was the highest scoring on-axis target, while p38-MAPK was the highest off-axis target. In addition, small cells displayed higher p38 activity and protein levels and spend more time in G1 compared to larger cells. Inhibition of p38-MAPK caused loss of the compensatory G1 length extension in small cells, resulting in faster proliferation, smaller cell sizes and increased size heterogeneity. Moreover, the evaluation of oversized yeast cells shared characteristics with old yeast cells and senescent human fibroblasts. Neurohr et al. reported that dilution of the cytoplasm causes the DNA to become limiting and perturb efficient MAPK response (Neurohr et al., 2019).

In a follow-up study, the off-axis targets from Liu et al. were categorized as either "dials" and "sensors". Compounds that influenced specific proteins that disproportionally affect cell size and G1 length (off-axis), are now further categorized depending on how perturbation of these specific proteins affects the relation between birth size and G1 length (cell size regulation). In the category dials are proteins that determine the target size for G1/S transition. When non-functional, these proteins cause an increased or decreased target cell size for G1 progression but do not impair the G1/S checkpoint. Sensors on the other hand, are proteins that facilitate the cell size checkpoint, perturbing these proteins results in an increase in cell size variance and a weakened relation between G1 length and birth size (Fig. 8).

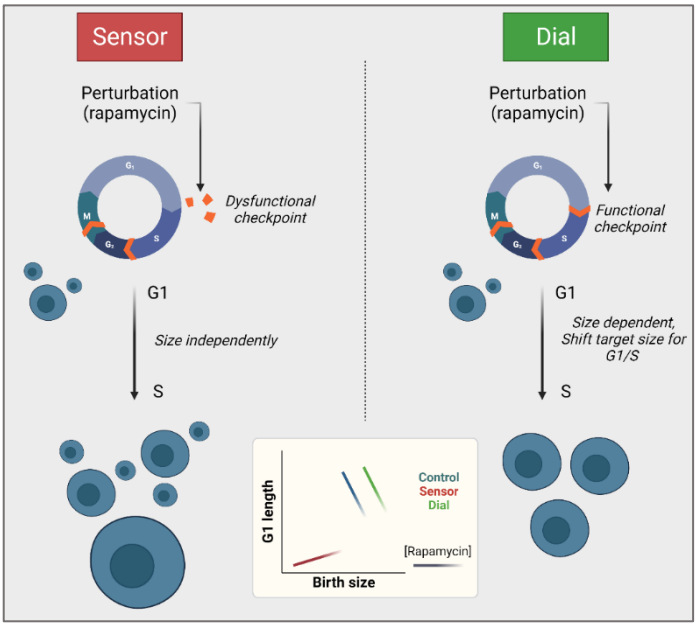


FIGURE 8: SCHEMATIC REPRESENTATION OF DIALS AND SENSORS.
RAPAMYCIN TREATMENT IMPAIRED GROWTH DURING G1 AND ALTERS CELL SIZE. HOWEVER, CELLS COMPENSATE FOR THE GROWTH IMPAIRMENT BY ELONGATING THEIR G1 PHASE. THIS IS THOUGHT TO BE FACILITATED BY 'SENSORS'. INACTIVATION OF SUCH A SENSOR DYSRUPTS THE G1/S CHECKPOINT AND CORRELATION BETWEEN G1 LENGTH AND CELL SIZE.
PROTEINS THAT AFFECT THE TARGET CELL SIZE FOR G1/S PROGRESSTION WHEN INACTIVATED ARE CALLED 'DIALS'. INACTIVATED DIALS CAUSE AN INCREASED TARGET CELL SIZE, BUT MAINTAIN A FUNCTIONAL G1/S CHECKPOINT. ADAPTED FROM (TAN ET AL., 2021), CREATED WITH BIORENDER.COM.

Interestingly, p38 was characterized as one of the strongest sensors, as p38 was selectively active in cells that were smaller than their target cell size (Liu et al., 2018; Tan et al., 2021) and chemical inhibition of p38 resulted in a dysfunctional G1/S checkpoint shown by an increase in cell size variation. Intriguingly, CDK4 was uncovered as one of the strongest dials. Consistent with the study from Edgar and colleagues (2018), CDK4 inhibition by Palbocyclib resulted in a larger target cell size. The unique relation between CDK4 and p38 was elucidated by epistasis tests, showing that p38 is usually kept active until a target cell size has been reached (Fig. 9). In the case of impaired CDK4 functioning, the target cell size becomes larger while p38 remains active even at sizes where it usually is not. It is thereby suggested that p38 is functioning as the sensor of the downstream CDK4 dial (Tan et al., 2021) (Fig. 9). Notably, the budding yeast p38 analogue Hog1 has also been associated with cell cycle regulation and coordination of CDK4 homolog Cln3 (O'Rourke & Herskowitz, 1998). However, no further research has specified a sensor/dial like interaction between the two proteins, to the best of knowledge.

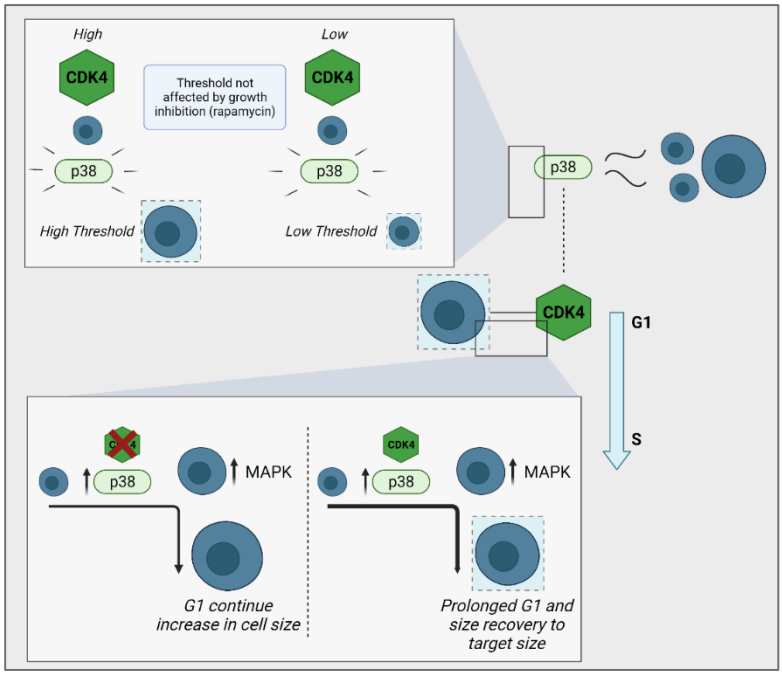


FIGURE 9: SCHEMATIC SUMMARY OF MAPK AND CDK4 SIZE REGULATION IN MAMMALIAN CELLS. AS P38 SENSES THE SIZE OF A CELL IN G1, IT INFLUENCES THE ACTIVITY OF CDK4 SUCH, THAT CDK4 ADJUSTS G1 LENGTH TO OBTAIN A TARGET CELL SIZE. IF A CELL IS SMALLER THAN THE CDK4 DETERMINED TARGET SIZE, P38 REMAINS ACTIVE AND PROLONGS G1 UNTIL THE TARGET SIZE HAS BEEN REACHED. INHIBITION OF CDK4 IMPAIRS TARGET SIZE DETERMINATION AND MAINTAINS P38 ACTIVITY SUCH THAT G1 CELLS DISPROPORTIONALLY INCREASE IN SIZE. CREATED WITH BIORENDER.COM.

LINKING GROWTH SIGNALS TO CELL SIZE REGULATION: YEAST AND MAMMALS

Naturally, growth signalling networks are connected to a cell's size regulation. The conserved target of rapamycin (mTOR) signalling network stimulates growth rate and increases cell size. In the absence of mTOR activity, a signalling cascade enhances Whi5 phosphorylation in yeast (Jorgensen & Tyers, 2004). Active mTOR however, controls ribosome biosynthesis rates which has been linked to the size threshold, via Ydj1 chaperone competition with yeast Cln3 (Aldea et al., 2017).

The cell size regulation models described above are mostly related to the strategy in which the cell elongates the G1 growth *period*. Alternatively, cells can compensate for their birth size by adjusting their growth *rate* during G1 (Fig. 1). Cln3 has been linked to the dynamic adjustment of growth rate due to its interactions with the chaperone protein Ydj1. In early G1, Cln3-Cdk1 is bound to the ER where it resides until Ydj1 facilitates translocation of the complex to the nucleus. As the chaperone is required for biogenesis (protein synthesis, translocation and ribosome synthesis) during G1, Ydj1 is less available for other processes such as the translocation of Cln3-Cdk1 (Vergé et al., 2007). Competition for the chaperone could prioritize growth instead of proliferation until a correct target size has been reached. Gene KO studies from proteins in the START regulatory network (Whi5, SBFs, *CLN2*, *CLN3, YDJ1* etc.) show that the linear correlation between size at birth and growth rate was lost when Ydj1 was inactivated (Ferrezuelo et al., 2012), suggesting that protein competition mechanisms may contribute to cell size regulation. Moreover, in mouse cells, the Ydj1 homolog Hsc70 was shown to associate with newly synthesized cyclin D, suggesting that protein hijacking mechanisms in cell size regulation are conserved in mammalian systems (Diehl et al., 2003).

Overall, the interactions between p38 and CDK4 provide direction for the identification of cell size sensors. Studies by Liu et al. (2018) and Tan et al. (2021) have showed that while p38 activity depends on cell size, CDK4 determines the target size for G1/S progression. As Rb concentrations are connected to size sensing and functions as downstream effector of CDK4, its role in this cell size homeostasis mechanism is yet to be determined.

CELL SIZE CONTROL IN PLANTS

Lastly, plant cell size homeostasis is uniquely investigated in a multicellular context. Specifically the shoot apical meristem (SAM) organ of *Arabidopsis thaliana* is favoured for these experiments as this organ actively proliferates and allows for lineage tracing. Following data from yeast and mammalian cells, plant research has revealed a similar link between cell cycle progression and cell size regulation (Jones et al., 2017). Evidence of size dependent cell cycle progression in plants has been observed for both G1/S and G2/M transition regulations. Recently however, it has been shown that the G1/S transition involving p27 functional analogue KRP4, plays a more prominent role in cell size maintenance (Fig. 4) (D'Ario et al., 2021).

KRP4 was shown to partition to daughter cells using DNA as internal scale, similar to the partitioning of Whi5 in yeast (D'Ario et al., 2021). Different to size constraining mechanisms in yeast and animals, cell sizes in plants were not corrected until after a few divisions, in the meristem periphery (Fig. 10) (Serrano-Mislata et al., 2015). Interestingly, overexpression of KRP4 resulted in 4-fold increases of meristem cell volumes in the centre. While cell sizes increased, cell growth rates remained stable and cell sizes were corrected in the periphery as a result of more frequent divisions, suggesting a role for KRP4 in the compensation of cell size during the G1/S transition. While KRP is described as a cell cycle inhibitor which is diluted during cell growth similar to mammalian Rb and yeast Whi5, it is molecularly distinct from the mammalian and yeast cell cycle regulators. Functionally, KRP4 influences cell cycle progression also rather indirectly compared to Whi5 and Rb, as KRP4 is a KIP related protein which inhibits Cdks, rather than being directly bound to transcription factors (D'Ario et al., 2021). Interestingly, recent developments in mammalian tissues indicate that KIP protein p27 plays a role in p38 activity required for cells to recover their original size (Liu et al., 2018). However, the compensation between cell size and proliferation is organ specific in plants and the balance between cell volume and cell cycle progression changes during the maturation of plant cells from the meristem (Schiessl et al., 2012; Tsukaya, 2008). The fact that the correlation between cell size and proliferation is organ specific in the multicellular model organism, indicates the complexity and variety of cell size regulation.

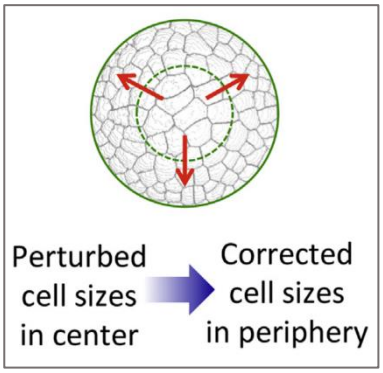


FIGURE 10: SIZE CORRECTION IN THE SAM PLANT CELLS ADJUST THEIR CELL SIZES IN THE PERIPHERY OF THEIR SAM. ADOPTED FROM (SERRANO-MISLATA ET AL., 2015)

DISCUSSION

This review has summarized the recent developments in research concerning cell size regulation. In general, cell size uniformity is maintained by balancing cell growth and G1 length, to resolve deviations on cell size at birth (Fig. 1). This balance seems to be universally regulated by either dilution of cell cycle inhibitors or accumulation of cell cycle activators as cells grow in G1. Naturally, there is variation between different studies, as different techniques are being used. A striking difference is noticeable in how cell size is being measured. Besides the measurement of cell size, growth conditions have been shown to have great impact on cell size regulations and results (Tan et al., 2021). Paradoxically, nutrient content is being exploited to study cell size regulation for a specific target cell size, as size regulation is thought to be more pronounced in poor nutrient conditions (Swaffer et al., 2021).

Not surprisingly, there is a debate on what type of cell size measurement is considered most accurate. Where some use the total protein mass of the cell (thought to scale with growth) (Litsios et al., 2019; Swaffer et al., 2021; Taheri-Araghi et al., 2015), others try to quantify cell volume (Dorsey et al., 2018; Litsios et al., 2019; Liu et al., 2018; Wang et al., 2009). Cell volume itself can be quantified using different techniques, of which live microscopy (Barber et al., 2020; Ferrezuelo et al., 2012; Tan et al., 2021) and Coulter Counter measurements (Dorsey et al., 2018; Litsios et al., 2019; Wang et al., 2009) are the main used. Cell volume measurements using live microscopy are executed using fluorescent labelling of the cell membrane, after which the cell volume is determined by mathematical calculation. A pitfall of fluorescence microscopy is that photo-bleaching could bias the results, for example this is being attributed to the dilution of Whi5 of which the concentration was also determined by fluorescent labelling (Dorsey et al., 2018). On the other hand, because protein mass is influenced by translation and carbon metabolism, the measurement of cell volume is thought to be more specific (M. Ginzberg et al., 2018). This suggestion is supported by Neurohr et al. (2019) showing that protein content was affected by a large cell size. However, Ginzberg's method of measuring cell volume is done by determining nuclear volume, as they find that it significantly coordinates with cell size (R=0.68). Although the relation between cell volume and nuclear volume may be significant, the relation they present between nucleus size and G1 length is not very strong as was shown in other studies (I. Conlon & Raff, 2003), indicating that this estimation of cellular volume might not be as accurate. Despite differences in determining fundamental size properties of cells, new techniques are being developed. Methods like Photo-Activated-Light-Microscopy (PALM) are cutting edge strategies to determine fluorescent protein concentrations, circumventing photo bleaching by combining multiple microscopy photos taken over time, resulting in super resolution live images. Perhaps this technology can give more accurate approximations of the size of an individual cell (Black et al., 2020).

Moreover, growth conditions have shown to alter cell size homeostasis, specifically in yeast. Volume at start displays large variability under different nutrient conditions and cells adjust their target size for G1/S progression accordingly (Ferrezuelo et al., 2012). Frequently, gene induction experiments are performed using galactose supplied medium, a relatively poor nutrient. Even though poor nutrient conditions can be motivated to pronounce size regulation (Schmoller et al., 2015), a certain cautiousness when using the extremes of nutrient content is required. It has been specifically investigated that Whi5 levels are affected by nutrient content, where low nutrient conditions are linked to high Whi5 levels (Qu et al., 2019). Furthermore, on a multicellular level, nutrient content may not appreciably affect maximal organismal size, but it can have a limiting effect on functional potential of an organ(ism) (Edgar, 2006). So when experiments on cell size regulation are predominantly executed using poor nutrient conditions, cellular fitness may be affected such that cell size regulation mechanisms may function in a state of stress rather than their usual homeostatic state.

CONCLUSION

Taken together, present techniques give new opportunities to more accurately determine cell size and its relation with cell cycle control factors. However, growth conditions need to be taken into account in designing cell size regulation research as nutrient contents can bias the results. Nevertheless, there is a trend visible in size regulation mechanisms across various model organisms. Although it is not clear why certain cell types have specific sizes, within a given cell type, an incredible cell size uniformity can be observed. Maintaining a cell type specific cell size is important for optimal function of the cell, as deviations from the target size indicate decreased cellular fitness. Cell size uniformity is thought to be determined by a balance in cell growth and the duration of the G1 phase of the cell cycle, such that cell size is maintained through divisions. Either the dilution of inhibitors, or accumulation of activators of G1 regulation is responsible for maintenance of the cell cycle checkpoint with regard to target size maintenance. As was understood from mammalian models, this target size is communicated by different 'dial' and 'sensor' proteins that transfer information of to the target size regulator.

BIBLIORAPHY

A. K. Abbas, A. H. Lichtman, & S. Pillai. (2016). *Cellular and Molecular Immunology* (9th ed.). Elsevier.

Alberts, B., Johnson, A., Walter, P., Raff, M., Lewis, J., & Roberts, K. (2008). *Molecular Biology of the Cell* (6th ed.). Garland Science.

Atay, O., & Skotheim, J. M. (2017). *Spacial and temporal signal processing and decision making by MAPK pathways*. https://doi.org/10.1083/jcb.201609124

Barber, F., Amir, A., & Murray, A. W. (2020). Cell-size regulation in budding yeast does not depend on linear accumulation of Whi5. *PNAS*. https://doi.org/10.1073/pnas.2001255117/-/DCSupplemental

Björklund, M., Taipale, M., Varjosalo, M., Saharinen, J., Lahdenperä, J., & Taipale, J. (2006). *Identification of pathways regulating cell size and cell-cycle progression by RNAi*. https://doi.org/10.1038/nature04469

Black, L., Tollis, S., Fu, G., Fiche, J.-B., Dorsey, S., Cheng, J., Ghazal, G., Notley, S., Crevier, B., Bigness, J., Nollmann, M., Tyers, M., & Ann Royer, C. (2020). *G1/S transcription factors assemble in increasing numbers of discrete clusters through G1 phase*. https://doi.org/10.1083/jcb.202003041

Blank, H. M., Callahan, M., Pistikopoulos, I. P. E., Polymenis, A. O., & Polymenis, M. (2018). *Scaling of G1 Duration with Population Doubling Time by a Cyclin in Saccharomyces cerevisiae*. https://doi.org/10.1534/genetics.118.301507

Boehlke, C., Kotsis, F., Patel, V., Braeg, S., Voelker, H., Bredt, S., Beyer, T., Janusch, H., Hamann, C., Gödel, M., Müller, K., Herbst, M., Hornung, M., Doerken, M., Köttgen, M., Nitschke, R., Igarashi, P., Walz, G., & Kuehn, E. W. (2010). Primary cilia regulate mTORC1 activity and cell size through Lkb1. *Nature Cell Biology 2010 12:11*, *12*(11), 1115–1122. https://doi.org/10.1038/ncb2117

Boniotti, M. B., & Gutierrez, C. (2001). *A cell-cycle-regulated kinase activity phosphorylates plant retinoblastoma protein and contains, in Arabidopsis, a CDKA/cyclin D complex*.

Cadart, C., Monnier, S., Grilli, J., Sáez, P. J., Srivastava, N., Attia, R., Terriac, E., Baum, B., Cosentino-Lagomarsino, M., & Piel, M. (n.d.). *Size control in mammalian cells involves modulation of both growth rate and cell cycle duration*. https://doi.org/10.1038/s41467-018-05393-0

Canovas, B., & Nebreda, A. R. (2021). Diversity and versatility of p38 kinase signalling in health and disease. *Nature Reviews Molecular Cell Biology*. https://doi.org/10.1038/s41580-020-00322-w

Chen, Y., Zao, G., Zahumensky, J., Honey, S., & Futcher, B. (2020). Differential Scaling of Gene Expression with Cell Size May Explain Size Control in Budding Yeast | Elsevier Enhanced Reader. *Molecular Cell*. https://reader.elsevier.com/reader/sd/pii/S109727652030160X?token=3866E2F7B50BC80CB1063CFA9B18659143F1E87BEBDAAAC3C54B2F515E046D135BEA287E7428ECD68B754734416C1796&originRegion=eu-west-1&originCreation=20211221104358

Conlon, I. J., Dunn, G. A., Mudge, A. W., & Raff, M. C. (2001). Extracellular control of cell size. *Nature Cell Biology 2001 3:10*, *3*(10), 918–921. https://doi.org/10.1038/ncb1001-918

Conlon, I., & Raff, M. (2003). Differences in the way a mammalian cell and yeast cells coordinate cell growth and cell-cycle progression. *Journal of Biology*, *2*(1), 1–10. https://doi.org/10.1186/1475-4924-2-7/COMMENTS

Constanzo, M., Nishikawa, J., Tang, X., Breitkreuz, K., Dewar, D., Rupes, I., Andrews, B., & Tyers, M. (2004). CDK Activity Antagonizes Whi5, an Inhibitor of G1/S Transcription in Yeast. *Cell Press*. http://www.cell.com/

D'Ario, M., Tavares, R., Schiessl, K., Desvoyes, B., Gutierrez, C., Howard, M., & Sablowski, R. (2021). Cell size controlled in plants using DNA content as an internal scale. *Science*, *372*(6547), 1176–1181. https://doi.org/10.1126/SCIENCE.ABB4348/FORMAT/PDF

de Bruin, R. A. M., Mcdonald, W. H., Kalashnikova, T. I., Yates, J., & Wittenberg, C. (2004). Cln3 Activates G1-Specific Transcription via Phosphorylation of the SBF Bound Repressor Whi5 fall into the G1-specific gene family (Cho et al they govern the events associated with cell cycle initiation, including DNA repli. *Cell*, *117*, 887–898.

Dhawan, S., Georgia, S., & Bhushan, A. (2007). Formation and regeneration of the endocrine pancreas. *Current Opinion in Cell Biology*, *19*(6), 634–645. https://doi.org/10.1016/J.CEB.2007.09.015

Dolznig, H., Grebien, F., Sauer, T., Beug, H., & Müllner, E. W. (2004). Evidence for a size-sensing mechanism in animal cells. *Nature Cell Biology 2004 6:9*, *6*(9), 899–905. https://doi.org/10.1038/ncb1166

Dorsey, S., Tollis, S., Cheng, J., Black, L., Notley, S., Tyers, M., & Royer, C. A. (2018). G1/S Transcription Factor Copy Number Is a Growth-Dependent Determinant of Cell Cycle Commitment in Yeast | Elsevier Enhanced Reader. *Cell Systems*. https://reader.elsevier.com/reader/sd/pii/S2405471218301881?token=891262896387851A3E7D6972FE23AADC8B778E898B6B6B8B19F7A4EE95781D15095AE2CEAE9827FCBD4A4B78F8ABB253&originRegion=eu-west-1&originCreation=20211221080622

Edgar, B. A. (2006). How flies get their size: genetics meets physiology. *Nature Reviews Genetics 2006 7:12*, *7*(12), 907–916. https://doi.org/10.1038/nrg1989

Facchetti, G., Chang, F., & Howard, M. (2017). Controlling cell size through sizer mechanisms | Elsevier Enhanced Reader. *Current Opinion in Systems Biology*. http://dx.doi.org/10.1016/j.coisb.2017.08.010

Farnier, C., Krief, S., Blache, M., Diot-Dupuy, F., Mory, G., Ferre, P., & Bazin, R. (2002). The Signaling Pathway for β1-Integrin/ERKs Is Involved in the Adaptation of Adipocyte Functions to Cell Size. *Annals of the New York Academy of Sciences*, *973*(1), 594–598. https://doi.org/10.1111/J.1749-6632.2002.TB04706.X

Farnier, C., Krief, S., Blache, M., Diot-Dupuy, F., Mory, G., Ferre, P., & Bazin, R. (2003). Adipocyte functions are modulated by cell size change: potential involvement of an integrin/ERK signalling pathway. *International Journal of Obesity 2003 27:10*, *27*(10), 1178–1186. https://doi.org/10.1038/sj.ijo.0802399

Ferrezuelo, F., Colomina, neus, Palmisano, A., Garí, E., Gallego, C., Csikász-nagy, A., & Aldea, martí. (2012). The critical size is set at a single-cell level by growth rate to attain homeostasis and adaptation. *Nature Communications*. https://doi.org/10.1038/ncomms2015

Fingar, D. C., Salama, S., Tsou, C., Harlow, E., & Blenis, J. (2002). Mammalian cell size is controlled by mTOR and its downstream targets S6K1 and 4EBP1/eIF4E. *Genes & Development*, *16*(12), 1472–1487. https://doi.org/10.1101/GAD.995802

Garmendia-Torres, C., Tassy, O., Matifas, A., Molina, N., & Charvin, G. (2018). Multiple inputs ensure yeast cell size homeostasis during cell cycle progression. *ELife*, *7*. https://doi.org/10.7554/ELIFE.34025

Ginzberg, M. B., Kafri, R., & Kirschner, M. (2015). Cell biology. On being the right (cell) size. *Science (New York, N.Y.)*, *348*(6236). https://doi.org/10.1126/SCIENCE.1245075

Ginzberg, M., Chang, N., Patel, N., Kafri, R., & Kirschner, M. W. (2018). *Cell size sensing in animal cells coordinates anabolic growth rates and cell cycle progression to maintain cell size uniformity*. https://doi.org/10.7554/eLife.26957.001

Guilherme, A., Virbasius, J. v., Puri, V., & Czech, M. P. (2008). Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nature Reviews Molecular Cell Biology 2008 9:5*, *9*(5), 367–377. https://doi.org/10.1038/nrm2391

Hall, M. N., Martin, R., & Thomas, G. (2004). *Cell Growth: Control of Cell Size* (1st ed.). Cold Spring Harbor Monograph.

Hartwell, L. H., Culotti, J., & Reidt, B. (1970). Genetic Control of the Cell-Division Cycle in Yeast, I. Detection of Mutants. *Proceedings of the National Academy of Sciences*, *66*(2), 352–359.

Heldt, F. S., Lunstone, R., Tysonid, J. J., & la Nová, B. (2018). *Dilution and titration of cell-cycle regulators may control cell size in budding yeast*. https://doi.org/10.1371/journal.pcbi.1006548

Johnston, G. C., Pringle, J. R., & Hartwell, L. H. (1977). *Coordination of Growth with Cell Division in the Yeast Saccharomyces Cerevisiae*.

Jones, A. R., Forero-Vargas, M., Withers, S. P., Smith, R. S., Traas, J., Dewitte, W., & Murray, J. A. H. (2017). Cell-size dependent progression of the cell cycle creates homeostasis and flexibility of plant cell size. *Nature Communications*. https://doi.org/10.1038/ncomms15060

Killander, D., & Zetterberg, A. (1965). Quantitative cytochemical studies on interphase growth: Determination of DNA, RNA and mass content of age determined mouse fibroblasts in vitro and of intercellular variation in generation time. *Experimental Cell Research*, *38*(2), 272–284. https://doi.org/10.1016/0014-4827(65)90403-9

Lengefeld, J., Cheng, C. W., Maretich, P., Blair, M., Hagen, H., McReynolds, M. R., Sullivan, E., Majors, K., Roberts, C., Kang, J. H., Steiner, J. D., Miettinen, T. P., Manalis, S. R., Antebi, A., Morrison, S. J., Lees, J. A., Boyer, L. A., Yilmaz, Ö. H., & Amon, A. (2021). Cell size is a determinant of stem cell potential during aging. *Science Advances*, *7*(46), 271. https://doi.org/10.1126/SCIADV.ABK0271/FORMAT/PDF

Litsios, A., E W Huberts, D. H., Terpstra, H. M., Guerra, P., Schmidt, A., Buczak, K., Papagiannakis, A., Rovetta, M., Hekelaar, J., Hubmann, G., Exterkate, M., Milias-Argeitis, A., & Heinemann, M. (2019). Differential scaling between G1 protein production and cell size dynamics promotes commitment to the cell division cycle in budding yeast. *Nature Cell Biology*. https://doi.org/10.1038/s41556-019-0413-3

Liu, S., Ginzberg, M. B., Patel, N., Hild, M., Leung, B., Li, Z., Chen, Y. C., Chang, N., Wang, Y., Tan, C., Diena, S., Trimble, W., Wasserman, L., Jenkins, J. L., Kirschner, M. W., & Kafri, R. (2018). Size uniformity of animal cells is actively maintained by a p38 MAPK-dependent regulation of G1-length. *ELife*, *7*. https://doi.org/10.7554/ELIFE.26947

Maciak, S., Bonda-Ostaszewska, E., Czarnoleski, M., Konarzewski, M., & Kozlowski, J. (2014). Mice divergently selected for high and low basal metabolic rates evolved different cell size and organ mass. *Journal of Evolutionary Biology*, *27*(3), 478–487. https://doi.org/10.1111/JEB.12306

Medina, E. M., Turner, J. J., Gordâ N, R., Skotheim, J. M., & Buchler, N. E. (2016). *Punctuated evolution and transitional hybrid network in an ancestral cell cycle of fungi*. https://doi.org/10.7554/eLife.09492.001

Miettinen, T. P., Pessa, H. K. J., Caldez, M. J., Fuhrer, T., Diril, M. K., Sauer, U., Kaldis, P., & Björklund, M. (2014). Identification of transcriptional and metabolic programs related to mammalian cell size. *Current Biology : CB*, *24*(6), 598–608. https://doi.org/10.1016/J.CUB.2014.01.071

Neurohr, G. E., Terry, R. L., van Werven, F. J., Holt, L. J., & Correspondence, A. A. (2019). Excessive Cell Growth Causes Cytoplasm Dilution And Contributes to Senescence. *Cell*, *176*. https://doi.org/10.1016/j.cell.2019.01.018

O'Rourke, S. M., & Herskowitz, I. (1998). The Hog1 MAPK prevents cross talk between the HOG and pheromone response MAPK pathways in Saccharomyces cerevisiae. *Genes & Development*, *12*(18), 2874–2886. https://doi.org/10.1101/GAD.12.18.2874

Pramila, T., Wu, W., Miles, S., Noble, W. S., & Breeden, L. L. (2006). *The Forkhead transcription factor Hcm1 regulates chromosome segregation genes and fills the S-phase gap in the transcriptional circuitry of the cell cycle*. https://doi.org/10.1101/gad.1450606

Qu, Y., Jiang, J., Liu, X., Wei, P., Yang, X., & Tang, C. (2019). Cell Cycle Inhibitor Whi5 Records Environmental Information to Coordinate Growth and Division in Yeast | Elsevier Enhanced Reader. *Cell Press*. https://reader.elsevier.com/reader/sd/pii/S2211124719312070?token=AB669A39B37F233AD7890DA3D4ABF39A3A0B5ADCDF8D6266BE065A6BE59ACDC7059E5A134ECF2FD4C9F0A8D6E79398C4&originRegion=eu-west-1&originCreation=20211220090536

Roskams, T. A., Theise, N. D., Balabaud, C., Bhagat, G., Bhathal, P. S., Bioulac-Sage, P., Brunt, E. M., Crawford, J. M., Crosby, H. A., Desmet, V., Finegold, M. J., Geller, S. A., Gouw, A. S. H., Hytiroglou, P., Knisely, A. S., Kojiro, M., Lefkowitch, J. H., Nakanuma, Y., Olynyk, J. K., … West, A. B. (2004). Nomenclature of the Finer Branches of the Biliary Tree: Canals, Ductules, and Ductular Reactions in Human Livers. *HEPATOLOGY*, *39*, 1739–1745. https://doi.org/10.1002/hep.20130

Satyanarayana, A., & Kaldis, P. (2009). Mammalian cell-cycle regulation: several Cdks, numerous cyclins and diverse compensatory mechanisms. *Oncogene*, *28*, 2925–2939. https://doi.org/10.1038/onc.2009.170

Schiessl, K., Kausika, S., Southam, P., Bush, M., & Sablowski, R. (2012). *JAGGED Controls Growth Anisotropy and Coordination between Cell Size and Cell Cycle during Plant Organogenesis | Elsevier Enhanced Reader*. http://dx.doi.org/10.1016/j.cub.2012.07.020

Schmoller, K. M., Turner, J. J., Koivomagi, M., & Skotheim, J. M. (2015). *Dilution of the cell cycle inhibitor Whi5 controls budding-yeast cell size*. https://www-nature-com.proxy.library.uu.nl/articles/nature14908.pdf

Sellam, A., Chaillot, J., Mallick, J., Tebbji, F., Albert, J. R., Cook, M. A., & Tyersid, M. (2019). *The p38/HOG stress-activated protein kinase network couples growth to division in Candida albicans*. https://doi.org/10.1371/journal.pgen.1008052

Serrano-Mislata, A., Schiessl, K., & Correspondence, R. S. (2015). Active Control of Cell Size Generates Spatial Detail during Plant Organogenesis. *Current Biology*, *25*. https://doi.org/10.1016/j.cub.2015.10.008

Skotheim, J. M., di Talia, S., Siggia, E. D., & Cross, F. R. (2008). Positive feedback of G1 cyclins ensures coherent cell cycle entry. *Nature*. https://doi.org/10.1038/nature07118

Spring, L. M., Wander, S. A., Zangardi, M., & Bardia, A. (n.d.). *CDK 4/6 Inhibitors in Breast Cancer: Current Controversies and Future Directions*. https://doi.org/10.1007/s11912-019-0769-3

Swaffer, M. P., Kim, J., Chandler-Brown, D., Langhinrichs, M., Marinov, G. K., Greenleaf, W. J., Kundaje, A., Schmoller, K. M., & Skotheim, J. M. (2021). Transcriptional and chromatin-based partitioning mechanisms uncouple protein scaling from cell size. *Molecular Cell*, *81*(23), 4861-4875.e7. https://doi.org/10.1016/J.MOLCEL.2021.10.007

Taheri-Araghi, S., Bradde, S., Vergassola, M., & Jun, S. (2015). *Cell-Size Control and Homeostasis in Bacteria | Elsevier Enhanced Reader*. http://dx.doi.org/10.1016/j.cub.2014.12.009

Tanaka, M., Kitanishi-Yumura, T., & Yumura, S. (123 C.E.). A "dynamic adder model" for cell size homeostasis in Dictyostelium cells. *Scientific Reports |*, *11*, 13742. https://doi.org/10.1038/s41598-021-92700-3

Tan, C., Ginzberg, M. B., Webster, R., Iyengar, S., Liu, S., Papadopoli, D., Concannon, J., Wang, Y., Auld, D. S., Jenkins, J. L., Rost, H., Topisirovic, I., Hilfinger, A., Derry, W. B., Patel, N., & Kafri, R. (2021). Cell size homeostasis is maintained by CDK4-dependent activation of p38 MAPK. *Developmental Cell*, *56*(12), 1756-1769.e7. https://doi.org/10.1016/J.DEVCEL.2021.04.030

Thorburn, R. R., Gonzalez, C., Brar, G. A., Christen, S., Carlile, T. M., Ingolia, N. T., Sauer, U., Weissman, J. S., & Amon, A. (2013). *Aneuploid yeast strains exhibit defects in cell growth and passage through START*. https://doi.org/10.1091/mbc.E12-07-0520

Traven, A., Whiteway, M., Sellam, A., Alonso-Monge, R., Correia, I., & Pla, J. (2017). The Hog1 MAP Kinase Promotes the Recovery from Cell Cycle Arrest Induced by Hydrogen Peroxide in Candida albicans. *Frontiers in Microbiology | Www.Frontiersin.Org*, *1*, 2133. https://doi.org/10.3389/fmicb.2016.02133

Tsukaya, H. (2008). *Controlling Size in Multicellular Organs: Focus on the Leaf*. https://doi.org/10.1371/journal.pbio.0060174

Tyers, M., Tokiwal, G., Nash, R., & Futcher, B. (1992). The Cln3-Cdc28 kinase complex of S.cerevisiae is regulated by proteolysis and phosphorylation. *The EMBO Journal*, *1*(5), 1–773.

Vergé, E., Colomina, N., Garí, E., Gallego, C., & Aldea, M. (n.d.). *Cyclin Cln3 Is Retained at the ER and Released by the J Chaperone Ydj1 in Late G1 to Trigger Cell Cycle Entry*. https://doi.org/10.1016/j.molcel.2007.04.023

Vergé, E., Colomina, N., Garí, E., Gallego, C., & Aldea, M. (2007). Cyclin Cln3 Is Retained at the ER and Released by the J Chaperone Ydj1 in Late G1 to Trigger Cell Cycle Entry. *Cell*. https://doi.org/10.1016/j.molcel.2007.04.023

Wang, H., Carey, L. B., Cai, Y., Wijnen, H., & Futcher, B. (2009). *Recruitment of Cln3 Cyclin to Promoters Controls Cell Cycle Entry via Histone Deacetylase and Other Targets*. https://doi.org/10.1371/journal.pbio.1000189

Willis, L., Refahi, Y., Wightman, R., Landrein, B., Teles, J., Huang, K. C., Meyerowitz, E. M., & Jönsson, H. (2016). *Cell size and growth regulation in the Arabidopsis thaliana apical stem cell niche*. https://doi.org/10.1073/pnas.1616768113

Xie, S., & Skotheim, J. M. (2021). Cell-size control: Chromatin-based titration primes inhibitor dilution. *Current Biology : CB*, *31*(19), R1127–R1129. https://doi.org/10.1016/J.CUB.2021.08.031

Zapata, J., Dephoure, N., MacDonough, T., Yu, Y., Parnell, E. J., Mooring, M., Gygi, S. P., Stillman, D. J., & Kellogg, D. R. (2014). PP2ARts1 is a master regulator of pathways that control cell size. *JCB*. https://rupress.org/jcb/article-pdf/204/3/359/1364039/jcb_201309119.pdf

Zatulovskiy, E., Zhang, S., Berenson, D. F., Topacio, B. R., & Skotheim, J. M. (2020). Cell growth dilutes the cell cycle inhibitor Rb to trigger cell division. *Science*, *369*(6502), 466–471. https://doi.org/10.1126/SCIENCE.AAZ6213/FORMAT/PDF

Zetterberg, A., Engström, W., & Dafgård, E. (1984). The relative effects of different types of growth factors on DNA replication, mitosis, and cellular enlargement. *Cytometry*, *5*(4), 368–375. https://doi.org/10.1002/CYTO.990050413

Zetterberg, A., & Larsson, O. (1991). Coordination between cell growth and cell cycle transit in animal cells. *Cold Spring Harbor Symposia on Quantitative Biology*, *56*, 137–147. https://doi.org/10.1101/SQB.1991.056.01.018