Sizing up to Divide: Mitotic Cell-Size Control in Fission Yeast

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Abstract

Schizosaccharomyces pombe is a good model to study cell-size control. These cells integrate size information into cell cycle controls at both the G1/S and G2/M transitions, although the primary control operates at the entry into mitosis. At G2/M there is both a size threshold, demonstrated by the fact that cells divide when they reach 14 μm in length, and also correction around this threshold, evident from the narrow distribution of sizes within a population. This latter property is referred to as size homeostasis. It has been argued that a population of cells accumulating mass in a linear fashion will have size homeostasis in the absence of size control, if cycle time is controlled by a fixed timer. Because fission yeast cells do not grow in a simple linear fashion, they require a size-sensing mechanism. However, current models do not fully describe all aspects of this control, especially the coordination of cell size with ploidy.

Contents

INTRODUCTION

> "*. . . . it is clear that the quantitative relations of chromosomes, nuclei, cytosomes and cell-aggregates offer a complex problem, and one that is incompletely solved.*"
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> Edmund B. Wilson, *The Cell in Development and Heredity*, 1925, p. 101

The quantitative relationships between chromosome number, nucleus size, and cell size were first documented by Boveri, Hertwig, and colleagues in the early 1900s and summarized by E.B. Wilson (1925). These researchers concluded that cell-size control was a complex and incompletely solved problem. This remains true today, nearly 100 years later. The early observations described by Wilson led to the idea of the karyoplasmic ratio, now referred to as the nucleocytoplasmic ratio. In 1962, Koch, Schaechter, and colleagues, working with bacterial cells, proposed that "cell size at division, or some closely related property, is under close physiological control" (Schaechter et al. 1962, p. 432). They also noted the small amount of variation in the size of cells at the time of division. Around the same time, Mitchison (1957) began working on cell growth using *Schizosaccharomyces pombe*. After his first publication describing the growth dynamics of single yeast cells, his lab began to study genetic control of the cell cycle, with particular attention to the control of cell size (Nurse et al. 1976). By this time, Killander & Zetterberg (1965a,b) had published work describing the relationship between cell mass and the initiation of DNA synthesis in mouse fibroblasts. Since then, there has been a plethora of studies on cell-size control in a

wide variety of organisms and cell types, but despite this work, molecular mechanisms for the coordination of cell growth with cell division have remained elusive.

This review focuses on the coordination of cell growth and division in one of the best-studied model systems, the single-celled fission yeast *S. pombe*, but discusses other cell types where appropriate.

Understanding Size Control

For a growing cell to successfully replicate, it must first make adequate amounts of protein, DNA, membranes, and organelles and also have an appropriate volume of cytoplasm in which to accurately segregate all of these components into two equally sized daughter cells. To achieve this, the cell must coordinate progression through the cell cycle with the accumulation of mass. Two approaches have been used to understand this coordination. The first is a more theoretical and abstract approach. This takes us back to the early studies carried out throughout the 1900s, which considered aspects of different size-control systems and tried to unpick the controls by predicting how different systems would behave. The second is a more mechanistic approach aimed at identifying the molecular mechanisms involved. This has been the focus of most recent work on cell-size control, but in this review we discuss both approaches.

We first introduce the concept of size homeostasis, which describes how cells sense their size and use this information to make corrections to ensure that they divide at the correct size. This is different than merely considering the threshold size at which cells are dividing, although this distinction is often not clearly made. (The term size threshold is used here to describe a certain size that the cell must reach for a cell cycle transition to occur.) We discuss whether or not a system requires a size-sensing mechanism, an argument that depends heavily on the growth pattern of the cells. Cells that accumulate mass in a linear fashion can maintain size homeostasis simply by controlling the cell cycle with a fixed timer (Brooks 1981). (The term timer describes a period of the cell cycle that is defined by a fixed amount of time.) We refer to this as passive size control because the cells are not directly measuring size, although they are able to control it indirectly through the timing of cell division. The alternative is an active size-sensing mechanism, which we argue most eukaryotic cells possess. We envisage such a size-sensing mechanism measuring the volume of the cell, a component related to cell volume, or an aspect of cell geometry and feeding this information into the cell cycle controls.

We begin by describing the model system of fission yeast and then discuss theoretical aspects of cell-size homeostasis before considering possible molecular mechanisms of cell-size control. We hope to give an idea of both where the field currently stands with regard to addressing this long-standing biological question and where some of the answers may lie.

S. POMBE AS A MODEL FOR CELL-SIZE CONTROL

Since the 1950s, the fission yeast *S. pombe* has been used as a model organism to study the cell cycle. Described by Murdoch Mitchison (1957), the fission yeast cell cycle is a convenient model system for several reasons. First, cells have a rigid cell wall imposing a regular geometry—a 4-μm-wide rod-shape—so cell volume can be easily calculated from length and width measurements (Mitchison 1957). Because cells grow by tip extension, with no significant change in cell width, the increase in cell length is proportional to the increase in volume and is also indicative of cell cycle stage. This growth pattern affects the shape that these cells adopt and thus the possible mechanisms to measure cell size. Second, cells have a rapid generation time (roughly 2 h in ideal growth conditions) (Mitchison 1957), so consecutive cycles can be easily observed in real

time under time-lapse microscopy. Third, *S. pombe* has a simple and well-studied cell cycle that requires only a single cyclin-dependent kinase, Cdc2, in complex with the B-type cyclin, Cdc13 (Coudreuse & Nurse 2010, Fisher & Nurse 1996, Hayles et al. 1994, Nurse & Bissett 1981). This simplifies genetic studies and, together with a gene deletion collection covering more than 90% of nonessential genes (Kim et al. 2010, Spirek et al. 2010), means that systematic screens for genes affecting cell cycle transitions can be carried out (Hayles et al. 2013, Navarro & Nurse 2012). Fourth, although there will inevitably be some cell-to-cell variation in growth rate, little variability in cell size exists at division (Fantes 1977). This suggests that cell size is actively sensed and regulated and makes *S. pombe* a good system to study the coordination of cell size with cell cycle progression.

The Fission Yeast Cell Cycle

The rod-shaped cells of *S. pombe* grow by tip extension and divide medially when they reach 14 μm in length (Mitchison & Nurse 1985). On entry into mitosis, growth ceases, as actin patches redistribute from the actively growing ends to the center of the cell to form the cortical ring for cytokinesis (Marks et al. 1986). After division, the two daughter cells initially resume growth from the old end—the previously growing end—until early G2, when growth is initiated at the new end created by the preceding cell division. This transition from monopolar to bipolar growth is termed NETO (new end take-off) (Mitchison & Nurse 1985). Both the actin and microtubule cytoskeletons play important roles in establishing a polarized axis to carry out this characteristic cycle of tip growth followed by medial fission (Hagan & Hyams 1988, Marks et al. 1986).

The orderly progression of cell cycle events is controlled by a single cyclin-dependent kinase, Cdc2 (Nurse & Bissett 1981, Nurse & Thuriaux 1980, Nurse et al. 1976), whose activity is low in G1 and increases through the cell cycle (Moreno et al. 1989). Cdc2 drives the onset of both S phase and mitosis; lower activity levels of this kinase drive the G1/S transition, with higher levels driving entry into mitosis (Coudreuse & Nurse 2010). Cdc2 associates with the B-type cyclin Cdc13 early in S phase, but its activity is kept low by inhibitory phosphorylation of Tyr15, which is carried out by Wee1 and Mik1 (Lundgren et al. 1991, Russell & Nurse 1987b). In late G2, Cdc25 removes this inhibitory phosphorylation and cells enter mitosis (Gould & Nurse 1989, Nurse 1990, Russell & Nurse 1986). Two known pathways act upstream of Wee1 and Cdc25 linking cell growth to the G2/M transition: the Cdr2-Cdr1 pathway and the stress response pathway (Breeding et al. 1998, Kanoh & Russell 1998, Petersen & Hagan 2005, Petersen & Nurse 2007, Russell & Nurse 1987a). The Cdr2-Cdr1 pathway has been described as a cell geometry-sensing pathway and includes the protein Pom1, which has been suggested to act as a size sensor (Martin & Berthelot-Grosjean 2009, Moseley et al. 2009). This is discussed in further detail later in this review. The stress response pathway consists of a MAP kinase pathway that regulates the recruitment of polo kinase to the spindle pole body and mitotic commitment. A number of genes have been found to act at the G2/M transition independently of these two pathways and Cdc2-Tyr15 phosphorylation, suggesting alternative pathways of cell growth input into the G2/M transition (Navarro & Nurse 2012).

Fission yeast has two size thresholds, one at the G1/S transition and a second at G2/M (Nurse 1975). In wild-type exponentially growing cells, only the G2/M threshold is relevant, as it results in all G1 cells being large enough to surpass the size threshold to enter S phase (Nurse & Thuriaux 1977) (**Figure 1**). G1 phase is therefore extremely short in wild-type cycling cells. All coordination of growth and cell cycle progression happens in G2, the length of which contracts or extends according to the size of the cell (Fantes & Nurse 1977). Cells harboring mutations in the *wee1* gene revealed this so-called cryptic G1/S sizer. (We use the term sizer to refer to a period of

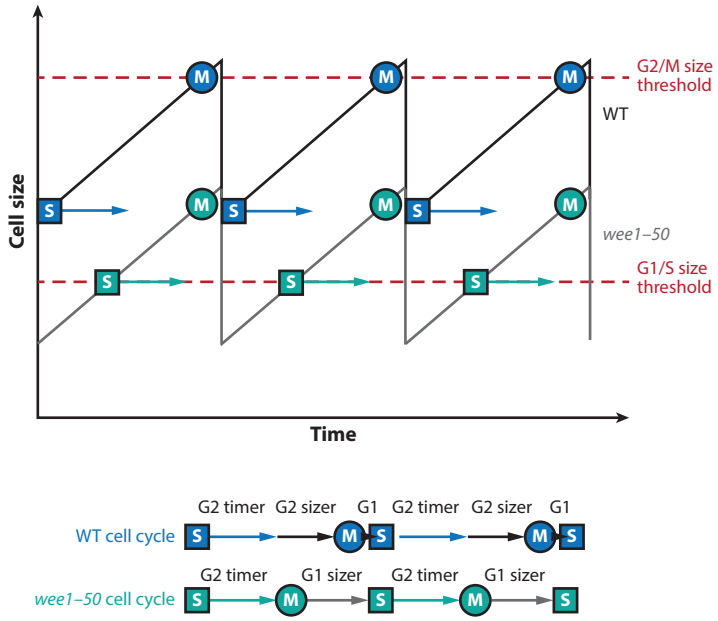

Figure 1

A not-to-scale schematic demonstrating the different cycles of wild-type and *wee1–50* cells (Nurse & Thuriaux 1977). For simplicity, linear growth is shown. WT cycling cells have a G2/M size threshold that results in G1 cells that already surpass the required size to enter S phase. G1 is therefore very short. G2 can be contracted or extended depending on the size of the cell. The G2 timer, sometimes referred to as the incompressible G2, is the minimum time that a cell must spend in G2. Temperature-sensitive *wee1–50* cells grown at the restrictive temperature do not have a G2/M size threshold. They enter mitosis immediately following the G2 timer period, and therefore G1 cells are below the minimum size requirement to enter S phase. G1 is extended for the cell to reach this G1/S size threshold. Abbreviations: M, mitosis; S, S phase; WT, wild type.

the cell cycle that is defined by the size of the cell.) Because these *wee1* cells lack a G2/M size control, G1 cells are smaller than the required size for entry into S phase. Thus, these cells have an elongated G1 phase to attain the correct size for the G1/S transition, followed by a timer from S phase to entry into mitosis (often referred to as the minimum incompressible G2 period) (Fantes & Nurse 1978, Nurse & Thuriaux 1977) (**Figure 1**).

Navarro & Nurse (2012) published the results of a near-genome-wide screen for new elements acting at the G2/M transition in fission yeast. This screen identified 18 genes that act negatively at mitotic entry, seven of which had not been previously described as having cell cycle regulatory roles. Eleven genes were shown to act through the known stress response and cell geometry-sensing pathways, and the remainder either acted through unknown pathways or were shown to be independent of CDK Tyr15 phosphorylation altogether (Navarro & Nurse 2012). This work opened up further investigation into as-yet-uncharacterized pathways acting on cell-size control for entry into mitosis.

This review focuses on the controls operating at G2/M. However, for many eukaryotic cells, including the budding yeast *Saccharomyces cerevisiae*, size control operates earlier in the cell cycle, at the G1/S transition (Johnston et al. 1977, Jorgensen & Tyers 2004, Turner et al. 2012). Therefore, both the G1/S and G2/M controls must contribute to a global cell size-sensing mechanism.

Unicellular Versus Multicellular Organisms

For unicellular organisms such as *S. pombe*, a major influence on cell size and shape is the surrounding environment. The cell surface area-to-volume ratio plays an important role in how efficiently the cell can take up the nutrients it needs; therefore, cell size is important for fitness (Smith 1925). For multicellular organisms, it is the surrounding cells and the position of a cell among others that influence its size and shape, as well as the cell's function in that particular tissue (Jorgensen & Tyers 2004, Marshall et al. 2012). Because this review focuses on fission yeast, we only briefly discuss cell-size control in multicellular organisms, but for a recent review of this area see Lloyd (2013).

CELL-SIZE HOMEOSTASIS

A size-control system for entry into mitosis has two characteristics: It determines the threshold size at which cells divide and the variability around this threshold. Cells of a certain type (be it of a single-celled organism or cells of a particular tissue within a multicellular organism) have a critical size at which division takes place. This is evident in the similarity of cell sizes at division within a particular population. The cell may be able to change this threshold size depending on signals from its surroundings, including nutrient availability, temperature, or external cues from surrounding cells. The variability around the size threshold shows how accurately the cells in the population are able to attain this critical cell size. A complete size-control system therefore requires the cell to first set a threshold size for entry into mitosis and then sense its size to make the appropriate corrections.

These corrections can be thought of as size homeostasis. The definition of homeostasis states that it requires a sensor to detect changes in the condition to be regulated, an effector mechanism that can vary that condition, and a negative feedback connection between the two. In relation to cell size, we can think of this system as a sensor to detect how big the cell is and how far this is from the required size, a mechanism that allows the cell to delay or promote entry into mitosis accordingly, and finally a continuous feedback mechanism that allows the correction to bring cell size to the required threshold without significantly overshooting.

It is important to distinguish between two possible causes of increased variability in cell size at division within a population. The first possibility is that each individual cell has a different size threshold that is accurately maintained, implying a loss of control at the population level as a result of clonal variability. The second is that all cells in the population try to attain a common size, but a problem in the control system results in weak homeostasis at the cellular level (Fantes 1981). When studying the cells of fission yeast, we typically work with clonal populations of single cells. Therefore, it is likely that increased variability results from the second possibility rather than the first.

The Importance of Studying Size Homeostasis

We argue that finding the genes involved in the homeostatic mechanism is key to solving the problem of how cells maintain accurate size control. We already have a good understanding of the key regulators involved in the decision to enter mitosis in fission yeast. These genes were discovered using mutations that generated size phenotypes, either advancing the cells into mitosis at a smaller size or lengthening G2 so that cells divided at a longer-than-wild-type size (Nurse 1975, Russell & Nurse 1986). These genes have a role in relaying size information into the cell cycle and could be involved in setting the threshold size for entry into mitosis. However, to find the

genes involved in sensing cell size, rather than setting or adjusting the threshold, size-homeostasis mutants should also be useful. We would expect populations of such mutants to display increased variability in cell size at division, as the ability to correct for deviations in size would be affected.

Three major experimental techniques allow us to study cell-size homeostasis. First, we can characterize the variation in size at division within a population of cells. A tight distribution implies that cells are able to accurately divide at the required threshold size, whereas a broader distribution implies sloppiness within this control system (at either the population or the cell level, as discussed above) (Fantes 1981). Second, we can observe the response of individual cells to the natural variation in cell size that occurs within a population of exponentially growing cells. If a cell divides to produce a daughter that is smaller than average, an effective homeostatic mechanism ensures that this small cell compensates in the subsequent cycle to attain the required size (Fantes 1977). The reverse is true for long cells, which should shorten their growth period to divide at the correct size. Third, but related to the second approach, we can actively perturb the size of cells within a population and measure how quickly they are able to return to the wild-type size. We hypothesize that cells defective in a size-sensing and correction mechanism take longer to achieve the correct size than cells with effective size homeostasis.

MECHANISMS OF CELL-SIZE CONTROL

Before beginning a discussion of size-sensing mechanisms, we consider whether or not a size-control mechanism is necessary for a population of cells to maintain size homeostasis.

No Size Control: A Passive Mechanism

A cell must control its size, but does this need to be an active process of sensing and signaling? The answer to this question lies in the growth pattern of the cell, and by growth we strictly refer to the accumulation of mass. Take a population of single cells growing in a linear manner, whereby the rate at which they accumulate mass is constant, regardless of their initial mass. If the time between successive divisions is maintained by a fixed timer, all cells will converge toward the population mean regardless of their initial size, as smaller cells will accumulate more relative mass in comparison with larger cells (**Figure 2*a***). The population will therefore maintain size homeostasis in the absence of size checkpoints, as long as an accurate timer is in place to control the timing between cell divisions (Brooks & Shields 1985, Conlon & Raff 2003). By contrast, if a population of cells is growing exponentially, large cells will grow faster than small cells and the population will diverge from a mean cell size. A fixed cell cycle timer will therefore be unable to maintain size homeostasis within a population of exponentially growing cells.

A major difficulty in testing this model has been the technical limitations in determining linear versus exponential growth. Neither budding nor fission yeast accumulate mass in a simple linear fashion for extended periods of time (Baumgartner & Tolic-Norrelykke 2009, Creanor & Mitchison 1982, Di Talia et al. 2007, Elliott & McLaughlin 1978, Godin et al. 2010, Mitchison 2003, Mitchison & Nurse 1985). Aside from measurements of several different growth parameters, evidence comes from the growth of the fission yeast temperature-sensitive size mutants *wee1–50* and *cdc25–22*. At a semirestrictive temperature, these cells have the same generation time as wild-type cells, showing that they double their mass in the same time frame (Thuriaux et al. 1978). Because *cdc25–22* cells are more than three times the size of *wee1–50* cells, they must add more mass per unit time (Nurse 1981). Thus, fission yeast cells are not growing in a simple linear fashion and cannot rely on a fixed timer to maintain size control.

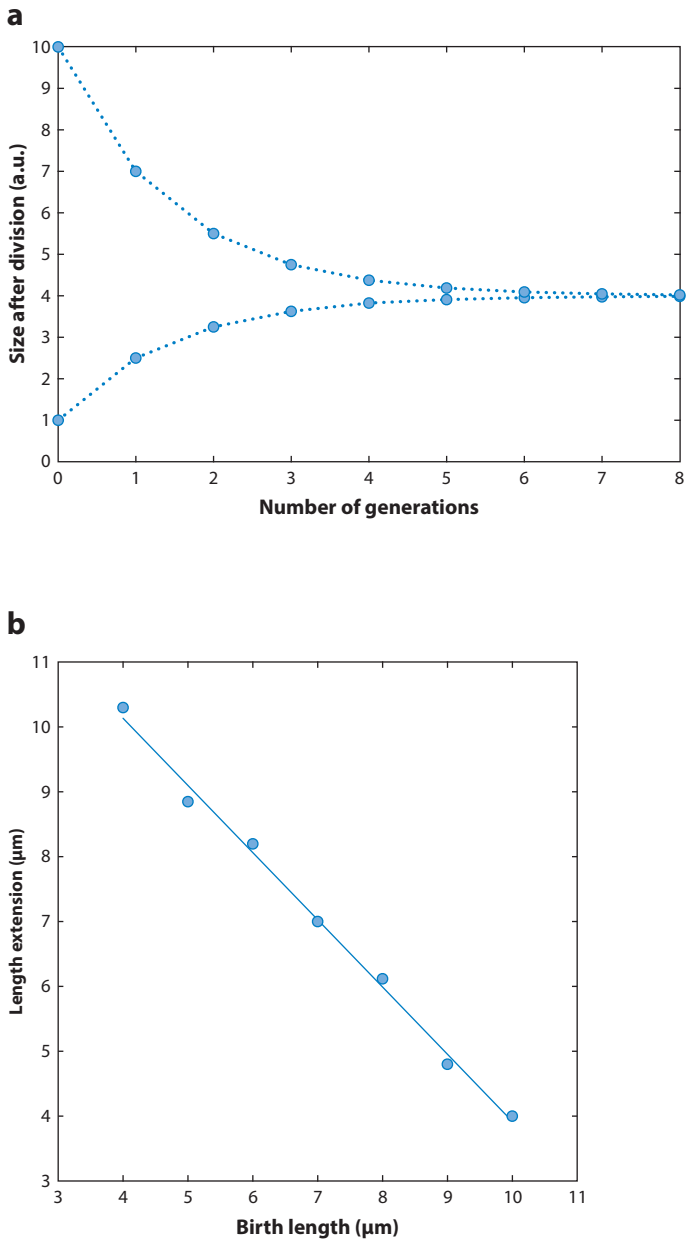

Figure 2

Theoretical graphs of (*a*) passive and (*b*) active size-control mechanisms. (*a*) A graph showing how linear growth and a fixed timer are able to maintain size homeostasis (Conlon & Raff 2003). If during each cell cycle a cell gains 4 units of mass regardless of its initial size, small cells will add more proportional mass than larger cells, and all cells will tend toward the average size of the population (which in this case is 4 units). (*b*) A population of cells exhibiting size control will show a negative relationship between birth length and length extension during the subsequent cycle (Fantes 1977). For example, a cell that is born small will extend more in the next cell cycle to attain the correct size (in this case 14 μm).

Multicellular Organisms: A Challenging Debate

The situation is less clear in cells of multicellular organisms. A cell's growth and division must be strictly controlled together with that of surrounding cells to form tissues and organs, and this may mask certain intracellular controls (Jorgensen & Tyers 2004). After Killander & Zetterberg's (1965a,b) pioneering work with mouse fibroblasts, many other groups working with a variety of cell types demonstrated that cell size can influence cell cycle timing, particularly at the G1/S transition (Darzynkiewicz et al. 1979, Dolznig et al. 2004, Gao & Raff 1997, Jorgensen & Tyers 2004, Kimball et al. 1971, Saucedo & Edgar 2002, Shields et al. 1978). However, many people have challenged the relationship between cell size and cell cycle timing (Baserga 1984, Conlon & Raff 2003, Conlon et al. 2001, Fox & Pardee 1970), and the question of growth has been at the center of the long-standing debate as to whether or not mammalian cells require size control (Brooks & Shields 1985, Sveiczer et al. 2004). Recent studies of mammalian cells using single-cell measurements of fixed steady-state populations and cells in microfluidic chambers have revealed size regulation at the G1/S transition, supporting those early findings of Killander and Zetterberg (Godin et al. 2010, Kafri et al. 2013, Son et al. 2012). We therefore conclude that a size-control system feeding into cell cycle controls, and not simply a fixed timer, maintains size homeostasis within most eukaryotic cell populations. Of course this conclusion must be modified for very large cells, such as dividing eggs, which are above the cell-size threshold. In these cases, progression through the cell cycle is likely to involve timer-like controls.

An Active Size-Control System

The alternative to indirect size control through a fixed cell cycle timer is what we shall call an active size-control system. This describes a mechanism of direct cell-size sensing. Evidence for the existence of an active size-control system in fission yeast, rather than simply a fixed timer, includes the small amount of variation in cell size at division within a population of cells. These cells divide at 14 μm in length with a coefficient of variation of roughly 6% (Sveiczer et al. 1996). Single-cell studies carried out in the 1970s showed that cells that are born small are able to correct for this in the subsequent cell cycle, such that a graph of birth length against length extension has a slope of approximately –1 (Fantes 1977) (**Figure 2*b***). This correction is realized through altered cycle times and not altered growth rates (Fantes 1977). Cells also rapidly recover cell size after perturbation through the altered timing of subsequent cell cycles (Mitchison & Creanor 1971). Cells arrested in S phase continue to grow and can reach sizes of 20–25 μm. When the cell cycle block is released, cells undergo rapid synchronous divisions and return to their wild-type size within two cell cycles (Wood & Nurse 2013). Such a rapid recovery suggests that a size-control system acts in these cells. In budding yeast, single-cell studies have shown that cells born smaller spend longer in G1 to allow them to grow until a critical size has been reached (Johnston et al. 1977), implying that these cells also maintain size homeostasis through an active size-control system. Therefore, we conclude that a size-sensing mechanism acts in G1 and G2 for fission yeast, although the control is normally cryptic in G1, and mainly in G1 for budding yeast (Turner et al. 2012).

Cell-Size Control Models

A size-sensing and correction mechanism must monitor the size of the cell and use this information to coordinate growth and cell cycle progression, so that at the next division, the cell is at the average size of the population. We envisage two main possibilities by which a cell could measure its size. First, in what we refer to as a volume or concentration model, a cell measures the volume of the

cytoplasm by the concentration of a particular molecule in the cell, which is proportional to cell volume. Second, in a geometry model, a molecular ruler measures some aspect of cell dimension such as length or surface area. We refer to this as a geometry model because the cell is probing an aspect of the cell's geometry. These two models will be discussed below, together with the current model for size control in fission yeast, which falls into the category of geometry sensing. This Pom1-gradient model, first proposed in 2009 by two independent groups (Martin & Berthelot-Grosjean 2009, Moseley et al. 2009), has recently been revisited, and variations of this model have since been proposed (Bhatia et al. 2014, Deng et al. 2014, Pan et al. 2014).

In the context of fission yeast, a model of size control must explain not only how wild-type, haploid, rod-shaped cells measure their size but also how size control is maintained in several other situations. First, a model must explain how size control works in cells of differing ploidy, in which, as we discuss below, an almost directly proportional relationship exists between the amount of DNA and cell size at division (Gregory 2001, Nurse 1985). Second, a model must account for multinucleate cells resulting from the failure or inhibition of cytokinesis; such cells display synchronous nuclear divisions within a single enlarged cytoplasm (Fantes et al. 1975, Neumann & Nurse 2007, Nurse et al. 1976). Here, nuclear divisions must be controlled through either a local signal from the surrounding cytoplasm or cortex or, perhaps, a diffuse global signal found throughout the entire cell. Third, a model must encompass shape mutants, which do not conform to the regular rod-shape seen in wild-type cells. In each of these situations, cells sense their size or volume and coordinate mitosis with cell growth; they must therefore be considered when proposing models for size control in this organism.

VOLUME/CONCENTRATION MODELS

Protein-Synthesis-Rate Sizer

In its simplest form, we can imagine a size-control mechanism whereby a protein that drives entry into mitosis accumulates at a rate proportional to the increase in cell mass. At a certain cell size, enough of this factor is present to initiate division. As the number of ribosomes in the cell is proportional to its size, the protein synthesis rate will correlate with cell size (Elliott & McLaughlin 1978). As long as this so-called sizer molecule has a high turnover rate, the amount of the factor in the cytoplasm will directly reflect protein synthesis and therefore the size of the cell (Turner et al. 2012). Such models were proposed many years ago to describe the size thresholds for division in bacteria and metazoans, as well as yeast (Brooks 1977, Donachie 1968, Fantes et al. 1975, Rossow et al. 1979, Unger & Hartwell 1976). A problem with this model is that as the cell grows, the increasing volume of the cytoplasm will dilute the effector molecule, resulting in it being present at a constant concentration. The cell therefore requires a fixed standard against which to measure the sizing factor, such as a component of the cell itself or a second effector molecule present in a fixed amount.

This is an issue with the current size-control model in budding yeast, in which the G1 cyclin Cln3, a dose-dependent activator of G1/S, is thought to act as a protein-synthesis-rate sizer (Cross 1988, Nash et al. 1988, Turner et al. 2012, Tyers et al. 1992). The concentration of Cln3 does not change as cells grow in G1, indicating that the increase in cell volume dilutes the increasing amounts of this protein (Tyers et al. 1993). It has been suggested that importing Cln3 into a nucleus of fixed volume would allow the cell to measure the absolute amount of the sizer (Futcher 1996). However, in budding and fission yeast, nuclear volume increases with cell volume to maintain a constant nuclear/cytoplasmic ratio throughout the cell cycle, which means that the nuclear volume does not remain fixed (Jorgensen et al. 2007, Neumann & Nurse 2007). Other suggestions have

involved titrating the sizer against the genome; this is discussed in more detail below, when we turn our attention to cell size and ploidy.

GEOMETRY MODELS

An alternative mechanism for measuring cell size is to measure geometrical parameters of the cell rather than the amount of a particular protein. Here, we use the word geometry to describe any property of cell shape that can be measured, such as length, width, or surface area (Moseley & Nurse 2010). In this case, we envisage a molecular ruler measuring an aspect of cell dimension.

The Pom1-gradient model proposed for fission yeast size control provides an example of such a sizer, with Pom1 acting as a molecular ruler through its concentration gradient, emanating from the cell tips (Martin & Berthelot-Grosjean 2009, Moseley et al. 2009). More recently, alternative, more complex models have been proposed that build on this original model (Bhatia et al. 2014, Deng et al. 2014, Pan et al. 2014). One of these models (Pan et al. 2014) suggests that cell surface area may be measured, but we can also imagine other structures in the cell, for example, internal membranes or microtubules, being probed by sizer molecules to feed size-related information into the cell cycle controls.

The Pom1 Gradient

Pom1 is a dual-specificity tyrosine-phosphorylation-regulated kinase (DYRK) (Bahler & Pringle 1998) that forms polar cortical gradients along the cell, peaking at the cell tips (Padte et al. 2006). The kinase was first described as providing positional information for both polarized growth and the site of septum formation (Bahler & Pringle 1998, Celton-Morizur et al. 2006, Padte et al. 2006). Pom1 has also been proposed to play a role in length sensing and the control of mitotic entry (Martin & Berthelot-Grosjean 2009, Moseley et al. 2009).

The Pom1 gradient is shaped by a combination of lateral diffusion through the cell cortex and autophosphorylation-dependent detachment (Hachet et al. 2011). Pom1 plasma membrane-binding is triggered by Tea4-dependent dephosphorylation by the phosphatase Dis2 (Hachet et al. 2011). As Tea4 is delivered to the cell ends by microtubules, Pom1 binding occurs at the cell tips. Pom1 then moves along the plasma membrane toward the cell middle and undergoes autophosphorylation to lower its lipid affinity, promoting release of Pom1 from the membrane (Hachet et al. 2011). Pom1 molecules diffusing at the plasma membrane also associate into and disassociate from clusters (Saunders et al. 2012). A two-state gradient involving these Pom1 clusters on the membrane has been suggested to serve as a mechanism to buffer against fluctuations in concentration levels. Along with time averaging, cluster formation could reduce the effects of inevitable fluctuations in gradient formation and may enable Pom1 to convey more precise spatial information (Saunders et al. 2012).

Pom1 as a Molecular Ruler

Evidence for the role of Pom1 in the control of cell size and mitotic entry was initially provided by two independent studies. Martin & Berthelot-Grosjean (2009) and Moseley et al. (2009) both showed that Pom1 acts as a dose-dependent inhibitor of the G2/M transition through the Cdr2-Cdr1-Wee1 pathway. Cdr2, Cdr1, and Wee1 are localized to a medial band of cortical nodes in interphase cells, and this localization is driven by Cdr2. Because Pom1 is found at the cell ends, it was hypothesized to provide spatial cues to the cortical nodes in the middle of the cell containing these cell cycle regulators. This idea was supported by data showing that Cdr2 spreads from its

tight band of cortical dots in *pom1Δ* cells and that Cdr2 is phosphorylated in a Pom1-dependent manner in vivo. Ectopic localization of Pom1 to the middle of the cell results in a delay in mitotic entry equivalent to the *cdr2Δ* phenotype, and this is dependent on an active Pom1 kinase domain (Martin & Berthelot-Grosjean 2009, Moseley et al. 2009). Quantification of the distribution of Pom1 and Cdr2 at the time showed that as cells progress through G2, Pom1 levels in the middle of the cell decrease, such that the proteins overlap in short but not long cells (Martin & Berthelot-Grosjean 2009, Moseley et al. 2009). These data will be discussed in more detail below. However, the original model proposed was as follows: In short cells, the Pom1 gradient from the cell ends overlaps with mitotic activators in the cortical nodes in the center of the cell. Pom1 phosphorylates and inactivates Cdr2. As cells increase in length through G2, the Pom1 concentration at the medial site becomes progressively lower, reaching a level at which it can no longer inhibit Cdr2. Wee1 is therefore inhibited, and active CDK drives the cell into mitosis (Martin & Berthelot-Grosjean 2009, Moseley et al. 2009). Nif1, a SEL1 repeat protein, is also implicated in this model because of its tip localization and the small-cell phenotype of *nif1Δ* cells (Martin & Berthelot-Grosjean 2009, Wu & Russell 1997).

Recently, Deng et al. (2014) have implicated another protein kinase, Ssp1, in this length-sensing model. They propose that Ssp1 phosphorylates and activates Cdr2 to promote mitotic entry but that Pom1 inhibits this activation in small G2 cells. Thus, they suggest that Cdr2 integrates the input signals from both the activating kinase, Ssp1, and the inhibitory Pom1 gradient (Deng et al. 2014).

Problems with the Pom1 Model

Despite these data supporting a gradient model, whereby Pom1 and possibly Nif1 act as molecular rulers to measure cell size, outstanding questions need to be addressed.

A quantitative analysis of the Pom1 protein, published after the initial model was proposed, showed a short decay length of the Pom1 gradient at the cell tips in relation to the length of the cell (Saunders et al. 2012). This study also found large cell-to-cell variability in both the intensity and distribution of the protein, as well as fluctuations in the cortical gradient over time on a single-cell level (Saunders et al. 2012). This means that changes in the protein gradient according to cell length may be less consistent than originally proposed and leads to questions regarding whether or not Pom1 could act as a reliable molecular ruler.

Another observation is that cells with *pom1* deletions demonstrate cell-size homeostasis (Wood & Nurse 2013). The role of Pom1 and Nif1 in size homeostasis was implied in the initial model but never directly tested. We have tested the involvement of these molecules using two approaches: an analysis of cell-size variability at mitosis and cell-size homeostasis studies on single cells (Wood & Nurse 2013). Wild-type fission yeast cells have a very low coefficient of variation in cell size at division, illustrating an effective size-control mechanism in these cells (Turner et al. 2012). The asymmetric division of *pom1Δ* cells (Bahler & Pringle 1998) creates large variation in cell size at birth; however, the level of variation is somewhat reduced by the time of cell division. This suggests that some size correction is taking place during a single cell cycle. Cells with *nif1* deletions have a wild-type size distribution at division (Wood & Nurse 2013). These data suggest that both deletion strains have some control present that minimizes the dispersion of sizes at division within a population. These data are further supported by direct homeostasis studies on single cells. Plotting birth length against length extension during the subsequent cycle shows a clear negative trend in wild-type, *pom1Δ*, and *nif1Δ* cells. This indicates that cells that are born small are able to sense this and alter the time spent and therefore mass added in G2 to reach the correct size. Long cells contract the time spent in G2 and thus extend less (Wood & Nurse

2013). According to the concept of the minimal G2 timer (the incompressible G2), in cells above a certain threshold size, the cycle cannot be compressed any further; thus, more than one cycle will be required to bring the cell back to average size (Fantes 1977). These single-cell studies suggest that neither Pom1 nor Nif1 are critical players in cell-size homeostasis, although both are clearly involved in setting the cell-size threshold.

In addition to the two observations above, the Pom1-gradient model is unable to explain size control in the three situations mentioned earlier: ploidy, multinucleate cells, and shape mutants. The model could be modified to take account of ploidy if gene copy number determined one of the components of the network. However, so far no such component has been identified (Marshall et al. 2012; J. Hayles, personal communication). With regard to multinucleate cells, the synchronous nuclear divisions suggest a global control mechanism throughout the continuous cytoplasm that is able to coordinate mitosis in each nucleus simultaneously (Fantes et al. 1975). Whether it is overall cell size or local nuclear cytoplasmic mass that is monitored has yet to be determined, and further insight into how this process fits with the Pom1-gradient model requires further investigation. Lastly, cell-shape mutants that do not conform to the regular rod-shape of wild-type cells cannot measure cell length through the Pom1 gradient alone, as these mutant cells divide at a constant surface area rather than a constant length (Pan et al. 2014).

Alternative Pom1 Models

Further evidence supporting an alternative mechanism for cell-size control came with improved cortical measurements of Pom1: No difference in medial Pom1 concentration between short and long cells was detected (Bhatia et al. 2014, Pan et al. 2014), as had been previously reported. It has been suggested that these are improved cortical measurements because previous measurements of Pom1 had been derived from total cellular fluorescence, which did not account for the exclusion of Pom1 from the nucleus (Bhatia et al. 2014, Pan et al. 2014, Saunders et al. 2012).

Although the amount of Pom1 in the very middle of the cell is similar in all cells, regardless of their size, the medial region of basal Pom1 widens linearly with increasing cell length. The Cdr2 domain also varies in width, node number, and intensity over the cell cycle (Bhatia et al. 2014, Pan et al. 2014), though the width increase of the Cdr2 domain is less than that of the basal Pom1 region (Bhatia et al. 2014). The result is that the degree of overlap between Pom1 and Cdr2, although very small, diminishes with increasing cell length (Bhatia et al. 2014). These data have led to several alternative length-sensing models.

The first model, most similar to the original, states that Pom1 inhibits Cdr2 activity at the edges of the node domain (Bhatia et al. 2014). Because the region of overlap between Crd2 and Pom1 decreases with increasing cell length, this provides a mechanism for length-dependent activation of Cdr2 and thus mitotic entry. This model is inherently noisy, resulting from the very small overlap of Cdr2 and Pom1, and also does not explain size control in diploids, in which the Pom1/Cdr2 overlapping region correlates poorly with cell length (Bhatia et al. 2014). Like the original model, it describes a length-sensing mechanism. The model is therefore, in its simplest form, unable to explain the behavior observed in multinucleate cells or mutant haploid cells of varying shapes. In these situations, the cell must be able to measure something other than its total length.

The second model postulates that cell size is measured through the ratio of polar Pom1-bound cortex to total cortex, a ratio that diminishes as the cell elongates (Bhatia et al. 2014). Cdr2 is inhibited close to the cell poles, where Pom1 concentration is highest. If the turnover of inhibitory phosphorylation is slow compared with the dynamics of Cdr2 on the cortex (also regulated by Pom1), inhibited Cdr2 accumulates in the middle of the cell. At a certain threshold dependent on cell length, too little of the cortex has Pom1 bound and therefore there is sufficient

active Cdr2 to drive mitotic entry (Bhatia et al. 2014). It is possible that this system could measure the surface area of the cortex rather than the length of the cell per se; therefore, this model could perhaps describe the behavior of both multinucleate cells and shape mutants, which cannot rely on a length-sensing mechanism alone. Another recent publication has proposed a surface area model that doesn't involve the Pom1 gradient directly but instead focuses on the medial cortical nodes (Pan et al. 2014). This model proposes that cells sense their size using Cdr2 to probe the entire surface area of the cell and relay this information to the medial cortex. Although both of these surface area-based models may be able to explain size control in cells of varying shapes and with more than one nucleus, they are still insufficient to explain the relationship between cell size and ploidy, as no component of these models has been linked to gene copy number.

A final possibility is that Pom1 does not act as a direct size sensor at all (Bhatia et al. 2014, Pan et al. 2014, Wood & Nurse 2013). The low levels of this kinase in the cell center may be sufficient to keep Cdr2 inactive and act as a constitutive buffer against mitotic commitment in response to other stimuli, such as the adaptation of cell length under stress (Bhatia et al. 2014). The higher levels of Pom1 at the cell tips could simply prevent nuclear division from occurring too near the cell ends, where there may not be sufficient space to allow complete separation of the two daughter nuclei (Wood & Nurse 2013). This is consistent with data suggesting that Pom1 is not the major size sensor and that another mechanism coordinates cell size with progression through the cell cycle (Wood & Nurse 2013).

THE PLOIDY PROBLEM

An important consideration in the understanding of cell-size control in fission yeast is that of ploidy. The almost direct proportionality between cell size at division and ploidy was observed as early as the 1950s, and it is considered an almost universal property of cell size in many cell types (Gregory 2001, Nurse 1985). Ploidy series in yeast have allowed us to observe this relationship directly (Mortimer 1958, Mundkur 1953) and conclude that ploidy determines cell size at division, which in turn determines the size of the daughter cells and their nuclei (Neumann & Nurse 2007). Cells are therefore able to feed information regarding their ploidy into the size-sensing mechanism. This is difficult to imagine in the current geometric size models described above.

Volume-Based Models Involving Ploidy

This is less of an issue if we turn to volume-based models for cell-size control. Two variations of this type of model could allow the cell to integrate information about DNA content into the size-control system. Either the cell could produce a factor according to ploidy, or it could measure a factor against ploidy (Marshall et al. 2012).

Making a factor according to ploidy could involve producing a transcript in a single pulse at a particular stage in the cell cycle. The amount of this transcript would be a direct function of DNA copy number. If the product of this critical transcript were stable and acted as a mitotic inhibitor, the fixed amount of protein produced from the pulse would be diluted as the cell grows. Below a critical protein concentration, and thus a critical cell size, entry into mitosis would be permitted (Fantes et al. 1975). The amount of the protein produced would increase with ploidy; thus, the cell would have to grow proportionally more before the critical concentration could be reached. A prediction of this model is that a heterozygous diploid with only one copy of such a gene would be the size of a haploid. No such gene has yet been described (Marshall et al. 2012; J. Hayles, personal communication). However, this may indicate redundancy within the system. In

fact, we can imagine a robust size-sensing mechanism involving multiple interacting factors acting together in a redundant manner.

The second volume-based model, which measures a factor against ploidy, could involve genomic titration (Donachie 1968, Fantes et al. 1975, Sompayrac & Maaloe 1973). Here, a protein is made at a constant concentration and said protein binds sites within the genome. As the cell grows, the absolute amount of protein increases and more genomic sites are bound (independent of protein concentration, which may remain constant, owing to the increasing volume of the cell during the cycle). The binding itself could drive division, so that as more sites are bound, the probability of entering mitosis increases. Alternatively, the protein in question may only be active in its free form; therefore, only once all the genomic sites are occupied will there be enough unbound protein to drive division. In both of these cases, an increase in ploidy would mean more sites on the DNA to bind. The cell would thus have to grow to a larger size before division is permitted. The first of these two possibilities has been suggested for budding yeast, in which the synthesis-rate sizer model involving Cln3 requires a fixed standard within the cell against which to titrate the G1 cyclin. It has been shown that Cln3 binds SBF binding sites across the genome, so it is possible that division is permitted only when a certain number of these sites are occupied (Wang et al. 2009). This would allow the cell to measure the amount of the cyclin, eliminating the problem of the constant concentration of Cln3 as the cell grows and also allowing input of ploidy into this size-sensing mechanism.

A third possibility can be envisaged that combines these two models. A factor produced according to ploidy could be used to titrate out the synthesis-rate sizer protein, which is present at a constant concentration. This would allow the cell to measure the comparative amounts of these two factors, a ratio which will change in a cell volume-dependent manner (Fantes et al. 1975).

The Pom1 Model and Ploidy

Turning to the Pom1 model in fission yeast, we must consider ploidy when proposing further adaptations to this size-control mechanism. Pom1 is haploinsufficient for cell size (Bhatia et al. 2014), showing that dosage of this protein is important for mitotic size control. However, heterozygous diploids are not reduced to the size of haploid cells; therefore, we conclude that Pom1 is not acting alone to measure ploidy directly. It could, however, be working in concert with other proteins involved in the proposed model. It would be interesting, for example, to investigate the effects of double and triple heterozygous mutants of Pom1, Cdr1, Cdr2, and Nif1 on the coordination of ploidy and cell size.

CONCLUDING REMARKS

Despite being such a long-studied and central question in cell biology, the mechanisms of cell-size control have remained elusive. The importance of understanding this problem has been overshadowed by the complexities involved in unpicking the intricate control system. Cell size can be influenced by many different factors, and for a long time experiments have been limited by technology. High-throughput imaging provides the means to readdress this problem.

The fission yeast *S. pombe* has served as an excellent model system for studying cell cycle transitions as a result of its regular geometry and tractable genetics. Though it had been thought that the major size-sensing system was identified in this organism following the two publications in 2009, the situation has once again become more complicated, with new data suggesting that an alternative mechanism is likely to operate either instead of or alongside the proposed Pom1 model.

We have argued here that many eukaryotic cells require a size-sensing mechanism and that investigating size homeostasis is a way to understand this problem. In our view, we must focus on the simple physiology of exponentially growing cells and their response to size perturbations, which can tell us a lot about the controls operating in these cells. Pom1 no doubt has a role in the G2/M transition in fission yeast, but it is not the whole story, and we should aim to identify other possible factors involved in sensing cell size and feeding this information back into the cell cycle controls. We may have imposed our own logic onto these cells—we, as scientists, use the length of these rod-shaped cells to study cell cycle progression, so it was a small leap to presume that the cells themselves may do the same. However, we should take a closer look at the data and ensure that we consider other possible mechanisms.

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The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

Bahler J, Pringle JR. 1998. Pom1p, a fission yeast protein kinase that provides positional information for both polarized growth and cytokinesis. *Genes Dev.* 12:1356–70

Baserga R. 1984. Growth in size and cell DNA replication. *Exp. Cell Res.* 151:1–5

Baumgartner S, Tolic-Norrelykke IM. 2009. Growth pattern of single fission yeast cells is bilinear and depends on temperature and DNA synthesis. *Biophys. J.* 96:4336–47

Bhatia P, Hachet O, Hersch M, Rincon SA, Berthelot-Grosjean M, et al. 2014. Distinct levels in Pom1 gradients limit Cdr2 activity and localization to time and position division. *Cell Cycle* 13:538–52

Breeding CS, Hudson J, Balasubramanian MK, Hemmingsen SM, Young PG, Gould KL. 1998. The *cdr2*$^{+}$ gene encodes a regulator of G2/M progression and cytokinesis in *Schizosaccharomyces pombe*. *Mol. Biol. Cell* 9:3399–415

Brooks RF. 1977. Continuous protein synthesis is required to maintain the probability of entry into S phase. *Cell* 12:311–7

Brooks RF. 1981. *The Cell Cycle*. Cambridge, UK: Cambridge Univ. Press

Brooks RF, Shields R. 1985. Cell growth, cell division and cell size homeostasis in Swiss 3T3 cells. *Exp. Cell Res.* 156:1–6

Celton-Morizur S, Racine V, Sibarita JB, Paoletti A. 2006. Pom1 kinase links division plane position to cell polarity by regulating Mid1p cortical distribution. *J. Cell Sci.* 119:4710–18

Conlon I, Raff M. 2003. Differences in the way a mammalian cell and yeast cells coordinate cell growth and cell-cycle progression. *J. Biol.* 2:7

Conlon IJ, Dunn GA, Mudge AW, Raff MC. 2001. Extracellular control of cell size. *Nat. Cell Biol.* 3:918–21

Coudreuse D, Nurse P. 2010. Driving the cell cycle with a minimal CDK control network. *Nature* 468:1074–79

Creanor J, Mitchison JM. 1982. Patterns of protein synthesis during the cell cycle of the fission yeast *Schizosaccharomyces pombe*. *J. Cell Sci.* 58:263–85

Cross FR. 1988. DAF1, a mutant gene affecting size control, pheromone arrest, and cell cycle kinetics of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 8:4675–84

Darzynkiewicz Z, Evenson DP, Staiano-Coico L, Sharpless TK, Melamed ML. 1979. Correlation between cell cycle duration and RNA content. *J. Cell. Physiol.* 100:425–38

Deng L, Baldissard S, Kettenbach AN, Gerber SA, Moseley JB. 2014. Dueling kinases regulate cell size at division through the SAD kinase Cdr2. *Curr. Biol.* 24:428–33

Di Talia S, Skotheim JM, Bean JM, Siggia ED, Cross FR. 2007. The effects of molecular noise and size control on variability in the budding yeast cell cycle. *Nature* 448:947–51

Dolznig H, Grebien F, Sauer T, Beug H, Mullner EW. 2004. Evidence for a size-sensing mechanism in animal cells. *Nat. Cell Biol.* 6:899–905

Donachie WD. 1968. Relationship between cell size and time of initiation of DNA replication. *Nature* 219:1077–79

Elliott SG, McLaughlin CS. 1978. Rate of macromolecular synthesis through the cell cycle of the yeast *Saccharomyces cerevisiae*. *PNAS* 75:4384–88

Fantes PA. 1977. Control of cell size and cycle time in *Schizosaccharomyces pombe*. *J. Cell Sci.* 24:51–67

Fantes PA, Grant WD, Pritchard RH, Sudbery PE, Wheals AE. 1975. The regulation of cell size and the control of mitosis. *J. Theor. Biol.* 50:213–44

Fantes PA, Nurse P. 1977. Control of cell size at division in fission yeast by a growth-modulated size control over nuclear division. *Exp. Cell Res.* 107:377–86

Fantes PA, Nurse P. 1978. Control of the timing of cell division in fission yeast. Cell size mutants reveal a second control pathway. *Exp. Cell Res.* 115:317–29

Fantes PA, Nurse P. 1981. Division timing: controls, models and mechanisms. In *The Cell Cycle*, ed. PCL John, pp. 11–33. Cambridge, UK: Cambridge Univ. Press

Fisher DL, Nurse P. 1996. A single fission yeast mitotic cyclin B p34^{cdc2} kinase promotes both S-phase and mitosis in the absence of G1 cyclins. *EMBO J.* 15:850–60

Fox TO, Pardee AB. 1970. Animal cells: noncorrelation of length of G1 phase with size after mitosis. *Science* 167:80–82

Futcher B. 1996. Cyclins and the wiring of the yeast cell cycle. *Yeast* 12:1635–46

Gao FB, Raff M. 1997. Cell size control and a cell-intrinsic maturation program in proliferating oligodendrocyte precursor cells. *J. Cell Biol.* 138:1367–77

Godin M, Delgado FF, Son S, Grover WH, Bryan AK, et al. 2010. Using buoyant mass to measure the growth of single cells. *Nat. Methods* 7:387–90

Gould KL, Nurse P. 1989. Tyrosine phosphorylation of the fission yeast *cdc2*$^{+}$ protein kinase regulates entry into mitosis. *Nature* 342:39–45

Gregory TR. 2001. Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. *Biol. Rev. Camb. Philos. Soc.* 76:65–101

Hachet O, Berthelot-Grosjean M, Kokkoris K, Vincenzetti V, Moosbrugger J, Martin SG. 2011. A phosphorylation cycle shapes gradients of the DYRK family kinase Pom1 at the plasma membrane. *Cell* 145:1116–28

Hagan IM, Hyams JS. 1988. The use of cell division cycle mutants to investigate the control of microtubule distribution in the fission yeast *Schizosaccharomyces pombe*. *J. Cell Sci.* 89(Pt. 3):343–57

Hayles J, Fisher D, Woollard A, Nurse P. 1994. Temporal order of S phase and mitosis in fission yeast is determined by the state of the p34^{cdc2}-mitotic B cyclin complex. *Cell* 78:813–22

Hayles J, Wood V, Jeffery L, Hoe KL, Kim DU, et al. 2013. A genome-wide resource of cell cycle and cell shape genes of fission yeast. *Open Biol.* 3:130053

Johnston GC, Pringle JR, Hartwell LH. 1977. Coordination of growth with cell division in the yeast *Saccharomyces cerevisiae*. *Exp. Cell Res.* 105:79–98

Jorgensen P, Edgington NP, Schneider BL, Rupes I, Tyers M, Futcher B. 2007. The size of the nucleus increases as yeast cells grow. *Mol. Biol. Cell* 18:3523–32

Jorgensen P, Tyers M. 2004. How cells coordinate growth and division. *Curr. Biol.* 14:R1014–27

Kafri R, Levy J, Ginzberg MB, Oh S, Lahav G, Kirschner MW. 2013. Dynamics extracted from fixed cells reveal feedback linking cell growth to cell cycle. *Nature* 494:480–83

Kanoh J, Russell P. 1998. The protein kinase Cdr2, related to Nim1/Cdr1 mitotic inducer, regulates the onset of mitosis in fission yeast. *Mol. Biol. Cell* 9:3321–34

Killander D, Zetterberg A. 1965a. A quantitative cytochemical investigation of the relationship between cell mass and initiation of DNA synthesis in mouse fibroblasts in vitro. *Exp. Cell Res.* 40:12–20

Killander D, Zetterberg A. 1965b. Quantitative cytochemical studies on interphase growth. I. Determination of DNA, RNA and mass content of age determined mouse fibroblasts in vitro and of intercellular variation in generation time. *Exp. Cell Res.* 38:272–84

Kim DU, Hayles J, Kim D, Wood V, Park HO, et al. 2010. Analysis of a genome-wide set of gene deletions in the fission yeast *Schizosaccharomyces pombe*. *Nat. Biotechnol.* 28:617–23

Kimball RF, Perdue SW, Chu EH, Ortiz JR. 1971. Microphotometric and autoradiographic studies on the cell cycle and cell size during growth and decline of Chinese hamster cell cultures. *Exp. Cell Res.* 66:17–32

Lloyd AC. 2013. The regulation of cell size. *Cell* 154:1194–205

Lundgren K, Walworth N, Booher R, Dembski M, Kirschner M, Beach D. 1991. mik1 and wee1 cooperate in the inhibitory tyrosine phosphorylation of cdc2. *Cell* 64:1111–22

Marks J, Hagan IM, Hyams JS. 1986. Growth polarity and cytokinesis in fission yeast: the role of the cytoskeleton. *J. Cell Sci.* 1986(Suppl. 5):229–41

Marshall WF, Young KD, Swaffer M, Wood E, Nurse P, et al. 2012. What determines cell size? *BMC Biol.* 10:101

Martin SG, Berthelot-Grosjean M. 2009. Polar gradients of the DYRK-family kinase Pom1 couple cell length with the cell cycle. *Nature* 459:852–56

Mitchison JM. 1957. The growth of single cells. I. *Schizosaccharomyces pombe*. *Exp. Cell Res.* 13:244–62

Mitchison JM. 2003. Growth during the cell cycle. *Int. Rev. Cytol.* 226:165–258

Mitchison JM, Creanor J. 1971. Induction synchrony in the fission yeast *Schizosaccharomyces pombe*. *Exp. Cell Res.* 67:368–74

Mitchison JM, Nurse P. 1985. Growth in cell length in the fission yeast *Schizosaccharomyces pombe*. *J. Cell Sci.* 75:357–76

Moreno S, Hayles J, Nurse P. 1989. Regulation of $p34^{cdc2}$ protein kinase during mitosis. *Cell* 58:361–72

Mortimer RK. 1958. Radiobiological and genetic studies on a polyploid series (haploid to hexaploid) of *Saccharomyces cerevisiae*. *Radiat. Res.* 9:312–26

Moseley JB, Mayeux A, Paoletti A, Nurse P. 2009. A spatial gradient coordinates cell size and mitotic entry in fission yeast. *Nature* 459:857–60

Moseley JB, Nurse P. 2010. Cell division intersects with cell geometry. *Cell* 142:184–88

Mundkur BD. 1953. Interphase nuclei and cell sizes in a polyploid series of *Saccharomyces*. *Experientia* 9:373–74

Nash R, Tokiwa G, Anand S, Erickson K, Futcher AB. 1988. The $WHI1^{+}$ gene of *Saccharomyces cerevisiae* tethers cell division to cell size and is a cyclin homolog. *EMBO J.* 7:4335–46

Navarro FJ, Nurse P. 2012. A systematic screen reveals new elements acting at the G2/M cell cycle control. *Genome Biol.* 13:R36

Neumann FR, Nurse P. 2007. Nuclear size control in fission yeast. *J. Cell Biol.* 179:593–600

Nurse P. 1975. Genetic control of cell size at cell division in yeast. *Nature* 256:547–51

Nurse P. 1985. The genetic control of cell volume. In *The Evolution of Genome Size*, ed. T Cavalier-Smith, pp. 185–96. Hoboken, NJ: Wiley

Nurse P. 1990. Universal control mechanism regulating onset of M-phase. *Nature* 344:503–8

Nurse P, Bissett Y. 1981. Gene required in G1 for commitment to cell cycle and in G2 for control of mitosis in fission yeast. *Nature* 292:558–60

Nurse P, Thuriaux P. 1977. Controls over the timing of DNA replication during the cell cycle of fission yeast. *Exp. Cell Res.* 107:365–75

Nurse P, Thuriaux P. 1980. Regulatory genes controlling mitosis in the fission yeast *Schizosaccharomyces pombe*. *Genetics* 96:627–37

Nurse P, Thuriaux P, Nasmyth K. 1976. Genetic control of the cell division cycle in the fission yeast *Schizosaccharomyces pombe*. *Mol. Gen. Genet* 146:167–78

Nurse P, Fantes PA. 1981. Cell cycle controls in fission yeast: a genetic analysis. In *The Cell Cycle*, ed. PCL John, pp. 85–98. Cambridge, UK: Cambridge Univ. Press

Padte NN, Martin SG, Howard M, Chang F. 2006. The cell-end factor Pom1p inhibits Mid1p in specification of the cell division plane in fission yeast. *Curr. Biol.* 16:2480–87

Pan KZ, Saunders TE, Flor-Parra I, Howard M, Chang F. 2014. Cortical regulation of cell size by a sizer cdr2p. *eLife* 3:e02040
Petersen J, Hagan IM. 2005. Polo kinase links the stress pathway to cell cycle control and tip growth in fission yeast. *Nature* 435:507–12
Petersen J, Nurse P. 2007. TOR signalling regulates mitotic commitment through the stress MAP kinase pathway and the Polo and Cdc2 kinases. *Nat. Cell Biol.* 9:1263–72
Rossow PW, Riddle VG, Pardee AB. 1979. Synthesis of labile, serum-dependent protein in early G1 controls animal cell growth. *PNAS* 76:4446–50
Russell P, Nurse P. 1986. *cdc25*$^{+}$ functions as an inducer in the mitotic control of fission yeast. *Cell* 45:145–53
Russell P, Nurse P. 1987a. The mitotic inducer *nim1*$^{+}$ functions in a regulatory network of protein kinase homologs controlling the initiation of mitosis. *Cell* 49:569–76
Russell P, Nurse P. 1987b. Negative regulation of mitosis by *wee1*$^{+}$, a gene encoding a protein kinase homolog. *Cell* 49:559–67
Saucedo LJ, Edgar BA. 2002. Why size matters: altering cell size. *Curr. Opin. Genet. Dev.* 12:565–71
Saunders TE, Pan KZ, Angel A, Guan Y, Shah JV, et al. 2012. Noise reduction in the intracellular Pom1p gradient by a dynamic clustering mechanism. *Dev. Cell* 22:558–72
Schaechter M, Williamson JP, Hood JR Jr, Koch AL. 1962. Growth, cell and nuclear divisions in some bacteria. *J. Gen. Microbiol.* 29:421–34
Shields R, Brooks RF, Riddle PN, Capellaro DF, Delia D. 1978. Cell size, cell cycle and transition probability in mouse fibroblasts. *Cell* 15:469–74
Smith HM. 1925. Cell size and metabolic activity in Amphibia. *Biol. Bull.* 48:347–78
Sompayrac L, Maaloe O. 1973. Autorepressor model for control of DNA replication. *Nat. New Biol.* 241:133–35
Son S, Tzur A, Weng Y, Jorgensen P, Kim J, et al. 2012. Direct observation of mammalian cell growth and size regulation. *Nat. Methods* 9:910–12
Spirek M, Benko Z, Carnecka M, Rumpf C, Cipak L, et al. 2010. *S. pombe* genome deletion project: an update. *Cell Cycle* 9:2399–402
Sveiczer A, Novak B, Mitchison JM. 1996. The size control of fission yeast revisited. *J. Cell Sci.* 109(12):2947–57
Sveiczer A, Novak B, Mitchison JM. 2004. Size control in growing yeast and mammalian cells. *Theor. Biol. Med. Modelling* 1:12
Thuriaux P, Nurse P, Carter B. 1978. Mutants altered in the control co-ordinating cell division with cell growth in the fission yeast *Schizosaccharomyces pombe*. *Mol. Gen. Genet.* 161:215–20
Turner JJ, Ewald JC, Skotheim JM. 2012. Cell size control in yeast. *Curr. Biol.* 22:R350–59
Tyers M, Tokiwa G, Futcher B. 1993. Comparison of the *Saccharomyces cerevisiae* G1 cyclins: Cln3 may be an upstream activator of Cln1, Cln2 and other cyclins. *EMBO J.* 12:1955–68
Tyers M, Tokiwa G, Nash R, Futcher B. 1992. The Cln3-Cdc28 kinase complex of *S. cerevisiae* is regulated by proteolysis and phosphorylation. *EMBO J.* 11:1773–84
Unger MW, Hartwell LH. 1976. Control of cell division in *Saccharomyces cerevisiae* by methionyl-tRNA. *PNAS* 73:1664–68
Wang H, Carey LB, Cai Y, Wijnen H, Futcher B. 2009. Recruitment of Cln3 cyclin to promoters controls cell cycle entry via histone deacetylase and other targets. *PLOS Biol.* 7:e1000189
Wilson EB. 1925. *The Cell in Development and Heredity*. New York: Garland
Wood E, Nurse P. 2013. Pom1 and cell size homeostasis in fission yeast. *Cell Cycle* 12:3228–36
Wu L, Russell P. 1997. Nif1, a novel mitotic inhibitor in *Schizosaccharomyces pombe*. *EMBO J.* 16:1342–50