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Geometric control of the cell cycle

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How do cells sense their own size and shape? And how does this information regulate progression of the cell cycle? Our group,[1] in parallel to that of Paul Nurse,[2] have recently demonstrated that fission yeast cells use a novel geometry-sensing mechanism to couple cell length perception with entry into mitosis. These rod-shaped cells measure their own length by using a medially-placed sensor, Cdr2, that reads a protein gradient emanating from cell tips, Pom1, to control entry into mitosis. Budding yeast cells use a similar molecular sensor to delay entry into mitosis in response to defects in bud morphogenesis. Metazoan cells also modulate cell proliferation in response to their own shape by sensing tension. Here I discuss the recent results obtained for the fission yeast system and compare them to the strategies used by these other organisms to perceive their own morphology.

Key words: cell cycle, cell polarity, concentration gradient, yeast, SAD kinase, pom1, cdr2, morphogenesis checkpoint, wee1

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In all living organisms, cell size is coordinated with cell division, but how does a cell know how big it is? Cell size homeostasis is particularly important in unicellular species, which directly use external nutrients to grow, to ensure that a constant cell size is maintained through thousands of generations. In metazoan organisms, cell size homeostasis is just as important, and involves additional non-cell autonomous layers of signaling that coordinate growth within tissues and organs.

In most, if not all, systems, cell size homeostasis appears to result from the dependence of certain cell cycle transitions on threshold cell size, ensuring a minimal cell size before entry to the next cell cycle stage. Conceptually, one can imagine that cell size is being sensed at multiple levels: by a measure of biosynthetic activity, a measure of cell or organelle mass or volume, or a geometric measure of cell size or shape. Over the years, many experiments have shown that all three types of measurements are being used.[3] While the rate of protein synthesis is generally thought to be a general measure of biosynthetic activity used to regulate key cell cycle transitions, organelle size—in particular the nucleo-cytoplasmic ratio—plays an important role during embryonic development. We have recently described a novel geometric measure of cell size in the fission yeast. Below, I discuss the evidence currently available to support the idea that a geometric sensing of cell size and shape contributes to cell size homeostasis in a range of organisms, comparing data in the fission yeast, budding yeast and metazoan systems.

Fission Yeast Cells: Control of Cell Size by a Spatial Concentration Gradient

Fission yeasts are rod-shaped cells that divide by binary fission to produce two equal-size daughters. In these cells, the main size control operates at the G_2/M transition. This transition is regulated by the cyclin-dependent kinase Cdk1/Cdc2:[4] during G_2, Cdk1 is maintained in an inactive state by the kinase Wee1, which phosphorylates it at Tyrosine 15. Dephosphorylation of this residue by the phosphatase Cdc25 activates Cdk1. Entry into mitosis thus depends on the balance between Wee1 and Cdc25 activities. In the absence of Wee1, Cdk1 activity is unrestrained and cells enter mitosis at a precociously small size, indicating that Wee1 is involved in size control. Of note, *wee1*

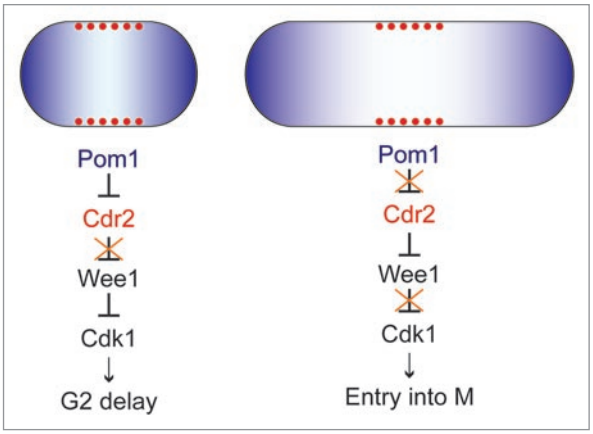

Figure 1. Model for how fission yeast cells coordinate cell length and division. Medial cortical nodes, coined the midsome, form cell cycle signaling centers containing the SAD kinase Cdr2 and the Cdk1-inhibitory kinase Wee1. Pom1 kinase, which negatively regulates Cdr2 forms a concentration gradient that emanates from the ends of the cells. In short cells, elevated medial concentration of Pom1 results in Cdr2 inhibition. This maintains the cells in the G_2 phase, during which polarized growth takes place. As the cells elongate, the medial concentration of Pom1 are reduced until it drops below a certain threshold at which Cdr2 is now active, allowing entry into mitosis.

deletion does not lead to mitotic catastrophe, essentially because of the existence of an additional size control at the G_1/S transition.[5]

Both our and Paul Nurse's groups have recently discovered that the DYRK-family kinase Pom1 is a novel upstream regulator of Wee1.[1,2] Pom1 mutant cells enter mitosis at a reduced cell size, and genetic and biochemical evidence suggest that Pom1 functions as a negative regulator of the SAD-family kinase Cdr2. Cdr2 and its homologue Cdr1, had been previously shown to inhibit Wee1.[6-10] The Pom1-Cdr2-Wee1-Cdc2 signaling pathway thus forms an inhibitory cascade that needs to be turned off for cells to enter mitosis.

While inhibitory cascades are well known modes of signaling, what is remarkable about this one is the intracellular localization of its components. While Cdk1 is largely nuclear,[11] Wee1 and Cdr2 are present in peri-nuclear cortical dots that form a broad band (coined the midsome[12,13]) at the geometric middle of the cell.[1,2,14] In contrast Pom1 forms gradients of highest concentration at cell poles and lowest concentration at the cell middle.[1,2,15] Both groups showed that the concentration of Pom1 at the cell middle varies during the cell cycle. In early G_2, short cells, where the two poles are close together, the medial concentration of Pom1 is higher than in late G_2, long cells. These data led to a model for how Pom1 serves to couple cell length with mitotic commitment (**Fig. 1**): in a short cell, medial Pom1 concentration is sufficiently high to inhibit Cdr2 and thus block Cdk1 activation, keeping the cell in a growth-active G_2 phase. As the cell grows, it reaches a length at which the medial Pom1 levels are below a threshold sufficient for significant Cdr2 inhibition. This leads to Wee1 inhibition, Cdk1 activation and entry into mitosis.

Several experiments were performed to test the importance of Pom1 gradients in this model. First, we showed that the effects of Pom1 on cell cycle regulation are exquisitely dose-dependent.[1] Indeed, less than 2-fold increase in Pom1 levels leads to cells dividing at significantly longer size. Second, both groups showed that alterations in the shape of Pom1 gradients (without increase in global Pom1 levels), by ectopically targeting Pom1 to the cell middle or by using mutants in which Pom1 fails to localize, result in higher medial Pom1 levels and G_2 delay.[1,2] These data thus strongly suggest that extremely small changes in Pom1 levels at the cell middle can tip the balance for or against entry into mitosis.

Gradients for measuring cell size. One outstanding question that these data raise is: How are Pom1 gradients shaped? Gradient formation may rely on active transport, localized translation or degradation, or simply diffusion and trapping of Pom1 at the cell tips. It is also unknown whether Pom1 is active at the cortex or also functions in the cytoplasm. The gradients shown are proposed to regulate mitotic commitment in the regular rod of wildtype fission yeast. But can different geometries be sensed? Are gradients used by other cell types to sense cell size and shape?

Concentration gradients have long been known to regulate development. Release of a diffusible morphogen from a specific site can produce an extracellular concentration gradient that provides positional information to cells.[16] More recently, it has been shown that intracellular processes are also regulated by gradients. The spatial organization of the microtubule cytoskeleton is regulated by gradients of diffusible regulatory molecules.[17] During cell cycle progression, a ran-GTP gradient provides a positional signal around chromosomes that organizes the mitotic spindle.[18] At the spindle midzone, Aurora B kinase generates a gradient of post-translational marks that provides spatial information for anaphase and cytokinesis.[19] Whether metazoan cells use intracellular gradients to sense size and shape is currently unknown, but mathematical modeling has shown that gradients have at least the potential to regulate signaling pathways both globally and locally in response to changes in cell size and shape.[20]

In bacteria, gradients are important to sense cell shape: In *E. coli* and *B. subtilis*, the MinC/D system forms oscillatory or static gradients from cell ends, respectively, that inhibit polymerization of FtsZ (the prokaryotic tubulin), promoting the medial localization of FtsZ rings for medial cell division.[21] In *C. crescentus*, the MipZ protein serves an analogous function: it binds to the partitioning protein ParB near origins of replications and localizes with these structures as DNA is segregated to the cell poles, from where it forms gradients. In turn these gradients define the cell middle for division by inhibiting polymerization of FtsZ at the poles.[22] MipZ thus links the temporal signal of DNA segregation with spatial regulation of cell division. It is interesting to note that extremely distant fission yeast

eukaryotes and several prokaryotes have evolved a similar gradient system to coordinate spatial and temporal aspects of cell division.

Coordination between cell morphogenesis and cell proliferation. Before this new cell cycle regulatory function was attributed to Pom1, Pom1 had been studied for over a decade for its role in cell morphogenesis. Indeed, *pom1* mutant cell were first discovered for their aberrant shape:[23] these cells display aberrant patterns of cell growth, misplace their site of growth to form kinked or T-shaped cells and misposition their site of division away from the cell middle. Thus, similar to the bacterial systems, Pom1 also reads cell shape to define the middle of the rod.

The site of division in fission yeast is positioned by complementary positive signal from the medially located nucleus and negative signals emanating from the cell poles. At the core of this regulation is the protein Mid1, whose localization to the midsome is under influence of both signals: Mid1 reads the nucleus position by shuttling in and out of the nucleus and is restricted from at least one cell pole by the action of Pom1.[15,24-26] For division site positioning, Pom1 plays two complementary functions. First, it functions in a midsome-independent manner to prevent septum formation at cell tips.[27] Second, it acts as an inhibitor to prevent the localization of midsome components at one cell end.[1,2,15,24,25] For this function, Pom1 again signals through Cdr2, which sits at the top of a midsome localization hierarchy and contributes to the localization of Mid1 to the midsome.[2,24] One singular observation is that both Cdr2 and Mid1 become asymmetrically localized around one entire cell half in *pom1* mutants. The signal that keeps these proteins off the second cell pole is currently unknown.

As both temporal and spatial controls of Pom1 over cell division converge onto Cdr2, one important question concerns the mechanism of Cdr2 inhibition. Does Pom1 phosphorylate Cdr2 to displace it from the cortex? Alternatively, does it phosphorylate Cdr2 to modify its activity? Is there a single Pom1 signal that controls Cdr2 for cell cycle progression and division site positioning, or do these represent distinct signals? One clue that the mechanisms may be distinct is that, while Pom1's role for cell cycle regulation is clearly dose-dependent,[1] such dose dependency has not been described for its spatial function. Dissection of the mechanism by which Pom1 regulates Cdr2 awaits mapping of the phosphorylation sites and dissection of their function.

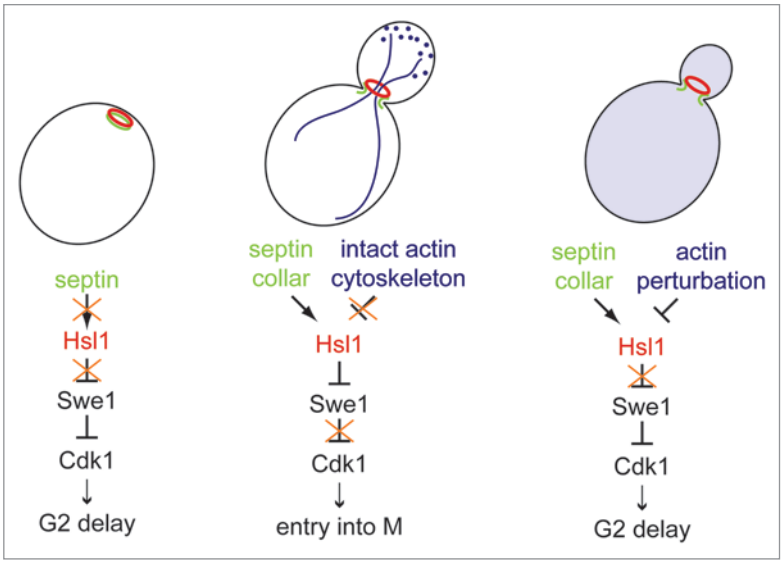

Figure 2. Model for how budding cells coordinate cell division with morphogenesis. The SAD kinase Hsl1 is recruited to the cortex by septins. In unbudded cells, Hsl1 remains inactive, such that Swe1 is not recruited at the cortex and promotes a G_2 delay by inhibiting Cdk1. Once the cell has budded, Hsl1 is activated and recruits Swe1 to degrade it, thereby promoting entry into M phase. Activation of Hsl1 may depend on the local geometry of septin organization, "reading" the shape of the bud neck. In budded cells, the morphogenesis checkpoint can be re-activated in response to perturbations in the actin cytoskeleton (displayed as diffuse).

Budding Yeast Cells: Control of Cell Shape by the Morphogenesis Checkpoint

Budding yeast cells also possess a geometry-sensing device. After a time of isotropic growth, cells choose a discrete cortical site where they initiate bud formation and subsequently direct all cell growth to the bud. The bud neck then represents the future site of cell division, and cells divide asymmetrically into a large mother cell and a smaller bud-derived daughter cell. Failure to form a bud blocks cell division and may lead to the formation of binucleate cells. To avoid this, budding yeasts have evolved a surveillance mechanism to delay entry into mitosis until the bud has been formed, which has been named the morphogenesis checkpoint (**Fig. 2**).

The cell cycle arrest caused by the morphogenesis checkpoint relies on Swe1, the *S. cerevisiae* Wee1 homologue. While this kinase is normally degraded to allow entry into mitosis, the morphogenesis checkpoint maintains high levels of Swe1. Degradation of Swe1 depends on the Cdr2-related SAD-family kinase Hsl1, which localizes to the bud neck in a septin dependent manner.[28,29] At the bud neck, Hsl1 is activated and, together with its partner Hsl7, recruits Swe1 and targets it for degradation.[29,30] When a bud fails to form, Hsl1 can be recruited to the septin cortex but remains inactive, such that Swe1 is not degraded and mitotic commitment is delayed. The morphogenesis checkpoint can also be activated upon environmental stresses once a bud has formed. Here again, Hsl1 localizes correctly to the bud neck, but is inactive and does not recruit Swe1.[30,31]

Exactly what Hsl1 is sensing is still being worked out. Hsl1 requires septins for its localization and recent work has proposed that Hsl1 may sense the local geometry of septin organization to distinguish pre-bud septin patch from bud neck collar arrangement of septin filaments.[31,32] After a bud has formed, the Hsl1-Swe1 system

also responds to stress insults and actin cytoskeleton disruption by delaying entry into mitosis. Since this response occurs only in small-budded cells, this led to the proposal that Hsl1 may be able to directly sense bud size.[33] However, recent work has provided firm evidence that it is the integrity of the actin cytoskeleton rather than bud size that is being sensed.[34]

Conceptually, the morphogenesis checkpoint in *S. cerevisiae* and the geometric control of cell length by the Pom1 system in *S. pombe* are very similar. There is an evident parallel in the logic of the two systems: in both cases a SAD kinase (Hsl1 and Cdr2) localizes at the future site of cell division. This SAD kinase acts as a sensor to monitor cell geometry and as a signal transducer to negatively regulate Wee1, which it recruits to this site. Although the mechanistic details of the negative regulation of Wee1 may be different in the two yeasts, these parallels strongly suggest a common evolutionary origin to these geometry-sensing machineries. In contrast, the signal sensed is likely to be different in the two yeasts. There is no evidence that there exist a Pom1-like gradient system in *S. cerevisiae*. Although such a gradient could conceptually be initiated at the bud tip to inactivate Hsl1 until sufficient bud size has been reached, this is difficult to reconcile with a direct role in sensing either septin or actin cytoskeleton. In *S. pombe*, the SAD kinase Cdr2 was also shown to act independently of septins.[14] Thus, although SAD-family kinases may be generally used as sensors of cell geometry, the particular signal they sense may be tailored to the specific shape of the cell monitored.

Metazoan Cells: Tension-Dependent Modulation of Cell Proliferation

Whether metazoan cells possess cell-intrinsic mechanisms to coordinate growth and division has been a long-standing question. Indeed, because somatic cells usually grow within multicellular tissues, and not as isolated cells, growth factors and mitogenic signals may simply be present in appropriate amounts in their environment. Very recent data obtained on mouse lymphoblasts now demonstrate that, at least in this cell type, cell growth is exponential (proportional to cell size).[35] This finding strongly suggests that metazoan cells have a powerful sizing mechanism to maintain their size during each cell cycle; small differences in the size of newborn cells would otherwise be quickly dramatically amplified.[35,36]

In contrast to the fungal cells described above, metazoan cells lack a cell wall to constrain the shape of the cell. Instead, cell shape is dictated largely by adhesion of the cell to its neighbors and the extracellular matrix. Because of the difficulty to separate environmental from cell-intrinsic changes in complex tissues, many experiments addressing the role of cell shape have been performed in vitro. Growing endothelial cells on micropatterned substrates demonstrated that cell shape governs whether a cell chooses to proliferate or die.[37] Subsequent studies performed either in flexible gels or on micropatterned substrates showed that cells perceive changes in extra- and intracellular tension (integrin-ECM binding and actin cytoskeleton organization) to modulate cell cycle progression, where higher tension generally favors cell proliferation.[38] It is however unclear whether and how cell shape per se is also sensed.

Conclusions

Yeast systems have led the way into understanding basic concepts in mechanisms that drive the cell cycle. It is clear that both fission and budding yeasts rely, at least in part, on a geometric measure to allow entry into mitosis. Our knowledge is much less advanced for metazoan cells, in which evidence for the existence of a cell-intrinsic size homeostasis system has just been uncovered. Details of how fission and budding yeast monitor their shape will need to be worked out and precisely compared, but for now, it looks like SAD kinases are key sensors in both systems. Since these two species have diverged about a billion years ago, one attractive, yet speculative possibility is that this family of kinases serves an evolutionarily conserved role beyond yeast in linking cellular geometry to cell proliferation.

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