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FREQUENCY CONTROL OF CELL CYCLE OSCILLATORS

Catherine Oikonomou[1,2] and **Frederick R. Cross**[1,*]
[1]The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA

SUMMARY

The cell cycle oscillator, based on a core negative feedback loop and modified extensively by positive feedback, cycles with a frequency that is regulated by environmental and developmental programs to encompass a wide range of cell cycle times. We discuss how positive feedback allows frequency tuning, how size and morphogenetic checkpoints regulate oscillator frequency, and how extrinsic oscillators such as the circadian clock gate cell cycle frequency. The master cell cycle regulatory oscillator in turn controls the frequency of peripheral oscillators controlling essential events. A recently proposed phase-locking model accounts for this coupling.

Oscillatory networks underlie the circadian clock, the beating of our hearts, and the cycle of cell division, which creates two cells from one, driving the reproduction and development of living systems. The characteristics and variations of this architecture found in biology have been excellently covered elsewhere (e.g. [1,2]). Here we focus on recent work on the variety of intrinsic and extrinsic factors that control the frequency of cell cycle oscillators.

In principle, simple genetic circuits can give rise to oscillations. For example, a negative feedback loop: X → R —| X can yield oscillations (X activates R, which inhibits X, so that R goes down, so that X goes back up…). Such a circuit requires significant non-linearity or a time delay to keep from rapidly settling to a constant steady state. An oscillator of this sort is thought to be the core of many eukaryotic cell cycles, such as the embryonic cell cycle in Xenopus [3] (Figure 1A).

Such negative feedback loops are also thought to be at the heart of multiple independent circadian systems (all operating with different biochemical machinery)[4–6]. Interestingly, a distinct three-negative-feedback ‘repressilator’ architecture has been constructed synthetically and shown to oscillate[7], but this architecture has not been observed in any natural system to our knowledge.

In known cases of negative-feedback oscillators, the ‘low X’ and ‘high X’ states are stabilized by positive feedback loops (or logically equivalent double negative feedback loops) (e.g.[8–10]). While these positive feedback loops are generally not essential for oscillations, empirically they appear to greatly increase the reliability of the oscillator. Theoretical comparison of negative-feedback-only and negative-plus-positive-feedback model versions of the cell cycle oscillator led to the conclusion that positive feedback dramatically increases the functional frequency range of the oscillator without significantly

*Correspondence: fcross@rockefeller.edu.
[2]coikonomou@rockefeller.edu

altering its amplitude; this may be a general feature of oscillatory systems[11•]. Positive feedback may be a source of elasticity to robustly accommodate a broad range of frequencies using the same fundamental oscillatory machinery.

The 'repressilator' architecture is relatively unstable, at least as initially constructed[7]; it is an interesting question whether stabilization of some of the state(s) by positive feedback would increase its robustness [11•].

In the eukaryotic cell cycle, the central negative feedback loop comprises the cyclin-CDK complex and its antagonist, an E3 ubiquitin ligase (Figure 1A) [12]. Positive feedback is added to the oscillator in multiple ways (Figure 1B) [8,10].

The cell cycle control system oscillates with a frequency that should be intrinsically determined by the timescales of protein synthesis, inhibition, and degradation. In most cells, though, the observed cell cycle frequency is highly variable, and much longer than the demonstrable minimum frequency (as an extreme example, the fly cell cycle in the early embryo can oscillate in ~10 minutes, while later somatic cells have cell cycle frequencies measured in hours or days). Recent work provides insight into how the frequency of the cyclin-CDK oscillator is controlled by intrinsic and extrinsic factors, as well as how the cyclin-CDK oscillator may modulate the frequency of a number of entrained oscillators controlling individual processes within the cell cycle.

Control of cell cycle frequency: cell size control and cell morphogenesis

A crucial modulator of the inherent frequency of the cell cycle is a set of controls designed to ensure the integrity of cell replication. These controls ensure that division does not occur under non-optimal conditions (e.g., insufficient cell size, errors in cell morphogenesis, incomplete DNA replication, DNA damage, and partially assembled mitotic spindles) by halting the cell cycle machinery at set points until requirements have been met[13]. Here we will limit our discussion to recent findings on the response of cell cycle frequency to cell size and cell morphogenesis.

In most cells (with the clear exception of embryonic cells resulting from the rapid division of very large fertilized eggs), cell growth is coordinated with cell division. In budding yeast, this coupling comes about because G1 cells of increasing size are increasingly likely to initiate 'Start', the activation of G1 cyclins that ultimately triggers the replicative cycle (Figure 2) [14,15]. Actual size 'measurement' was recently proposed to operate through direct binding of the most upstream G1 cyclin, Cln3, to the target sequence of a critical G1/S-regulatory transcription factor, with Start occurring upon titration of these sites by Cln3 – remarkably, increases in the genomic copy number of the transcription factor binding site can increase cell size at Start in a Cln3-dependent manner[16••]. This proposal directly anchors size measurement to a comparison between size, as reflected by Cln3 protein levels, and the fixed genomic content of specific sites.

Budding yeast exhibit asymmetric cell division, with larger mothers and smaller daughters. Interestingly, the regulation of Start by cell size was recently shown to be involved with this asymmetry through cell-type (mother-daughter) transcriptional control of *CLN3*. The unusual budding mode of division means that mother cells retain all their size from previous cell cycles and are larger than daughters – mothers have already 'passed' size control during their previous cell cycle. Correspondingly, mothers exhibit a pulse of *CLN3* transcription immediately upon cell division which may drive a prompt and largely size-independent Start; daughters lack this pulse and hence are much more tightly size-controlled[17•,18]. Therefore mother cells exhibit fairly regular and rapid cell divisions, compared to the much

slower division time of daughters, with the difference largely due to the need for extended cell growth in daughters.

Once Start is initiated, there is a handoff to a size-independent transcriptional positive feedback loop, which likely makes Start irreversible, and leads to near-simultaneous activation of hundreds of genes [19•,20•]. This is another example of the use of positive feedback loops to stabilize regulatory transitions, independent of those cited above; yet another example of this common regulatory theme was reported at the metaphase-anaphase transition in mitosis, where a positive feedback loop controlling the activation of separase (the protease responsible for separating sister chromatids) ensures a rapid and switch-like dissolution of cohesion[21•].

In fission yeast, in contrast to the findings in budding yeast, size gates the cell cycle at the point of mitotic entry, through an interesting mechanism that may use the cortex of the cell as a checkpoint scaffold. In these rod-shaped cells, an inhibitor of mitosis is localized to the cortex at the two ends of the rod. As the cell elongates and the ends move apart, the concentration at the middle of the cell (where the nucleus is located) drops; it is proposed that when this concentration is sufficiently low, mitotic entry is permitted. This mechanism ultimately operates through regulation of CDK inhibitory phosphorylation by Wee1, the same circuitry that controls positive feedback in the Xenopus embryo system[22•,23•] (Figure 2).

The apparent differences in size control between budding and fission yeast may not be absolute, since fission yeast were long ago reported to have ‘cryptic’ G1 size control (hidden by the stringency of the G2 size control, which results in all cells being bigger than the proposed G1 size control at birth)[24]. Similarly, it has been proposed that Wee1-dependent CDK regulation may control a G2 size control step in budding yeast[25], although the extent to which this mechanism controls cell size has raised some controversy. An alternative view is that in budding yeast, the circuitry is tied to a morphogenetic checkpoint blocking mitosis until bud morphogenesis is complete[26]. It may be that ultimately Wee1-dependent size control and a Wee1-dependent morphogenetic checkpoint are not that contradictory, since both views assert that the Wee1 system responds to some feature of bud morphogenesis (size and/or structure); indeed, this is reminiscent of the fission yeast mechanism described above, in which cell size and morphogenesis (a ‘long-enough’ rod-shaped cell) are integrated to control Wee1. Kinases involved in the fission yeast regulatory system are homologous to a budding yeast kinase involved in the Wee1-dependent morphogenetic checkpoint, emphasizing this parallel[27].

A similar growth-measuring mechanism controls cytokinesis in the bacterium *Bacillus subtilis*, where inhibitory proteins are localized to the pole; when their concentration at the middle of the cell drops due to increased distance from the poles, assembly of the cytokinetic ring begins[28][29].

Use of the cell wall as a scaffold is a common theme of bacterial cell cycle control. Perhaps most fundamentally, tethering chromosomes to the membrane on either side of the division plane ensures equal segregation upon division[30]. In the asymmetrically dividing bacterium *Caulobacter crescentus*, cell cycle regulatory proteins are commonly localized to one pole or the other, to activate or degrade proteins specifically[31]. Recently, polar localization has been shown to temporally control the auto-activation of a central kinase[32,33•]. This auto-activation occurs upon localization to one pole of the cell (and thus increased concentration). The protein responsible for recruiting the kinase to that pole is present only upon initiation of DNA replication, providing a temporally-restricted dock which ensures DNA replication

has begun before later events can be triggered (including silencing of the origin to prevent DNA re-replication)[34•].

Entrainment of the cyclin-CDK oscillator and other oscillators

Extrinsic oscillators

The cell cycle is not the only oscillatory system present in cells, and its frequency appears to be linked to these other cycles. Metabolic cycles regulate modes of nutrition in single-celled organisms under nutrient-limited conditions [35,36] and there is evidence in budding yeast that, under chemostat conditions where cells undergo synchronized oxido-reductive metabolic cycles, DNA replication may be restricted to the reductive portion of the cycle, perhaps to limit damage from reactive oxygen[37–39].

Additionally, circadian rhythms influence cell cycle frequency, although this restriction operates differently in different systems. In many proliferating mammalian tissues and in zebrafish embryos, S-phase is gated[40–43], by an unknown mechanism, perhaps in order to prevent DNA damage from UV exposure. In mouse fibroblasts and regenerating liver cells, though, the gating occurs not at a point before S-phase, but rather at a later point, prior to division[44,45]. In this case, core components of the circadian oscillator directly control the transcription of Wee1, the CDK inhibitor, and thus the timing of mitosis[44].

Recently, work in the cyanobacterium *Synechococcus elongatus* indicates that circadian gating in this organism occurs at the time of cytokinesis[46,47]. It was suggested that this protects the fidelity of inheritance of circadian clock components[47].

The variety of gating mechanisms in different organisms suggests multiple roles for this input. Perhaps different control points reflect different selective pressures (e.g. circadian clock synchronization in daughter cells vs. high fidelity DNA replication). Presumably, metazoan or plant cells have different requirements than single-celled organisms, and exposure to light-dark cycles will similarly vary.

Intrinsic peripheral oscillators

The cell cycle has so far been described as the oscillation of a master regulatory cyclin-CDK activity circuit, which triggers events at the correct time. As described above, external oscillators can modulate the frequency of this cycle. But even when the cyclin-CDK oscillator is locked at a constant high but physiological level, the cell cycle progresses with surprising efficiency[48]. These observations may be explained by the functioning of another class of oscillators whose frequency is set by the master cyclin-CDK oscillator. Continuing cycles of centrosome duplication occur in the absence of cell cycle progression in sea urchins, *Drosophila*, *Xenopus*, yeast, and CHO cells[49–54•]. In budding yeast, additional independent oscillators have been observed. Growth polarization (responsible for producing the bud) exhibits periodic cycles in the absence of cyclin-CDK activity and cell cycle progression[55]. Recently, an oscillator controlling the activity of the mitotic phosphatase Cdc14 has been shown to freely cycle at locked high levels of cyclin-CDK activity (Figure 3A)[56••]. In addition, almost 70% of cell-cycle regulated genes continue their periodic and timely expression in the absence of S/G2/M cyclin-CDK activity or cell cycle progression, suggesting an independent transcriptional oscillator[57•].

These events with the potential to oscillate in the absence of cyclin-CDK activity oscillations are nevertheless tightly controlled to occur once-per-cell-cycle in normally cycling cells, even at vastly different cell cycle timescales. How does the cyclin-CDK activity oscillator (almost surely the primary driver[58]) coordinate these ‘peripheral’ oscillators? Recent experimental and theoretical work suggests the hypothesis that

coordination may occur through a phase-locking mechanism, in which the master cyclin-CDK oscillator can force peripheral oscillators, either advancing or delaying them within a sensitive period, to ensure that they fire once and only once in each execution of the driving (CDK) cycle[56••] (Figure 3B–C). (This mechanism is exactly analogous to light entrainment of otherwise free-running circadian oscillators to once per light-dark cycle.) Such a phase-locking model can account quantitatively for once-per-cell-cycle Cdc14 activation, based on the experimentally measured strength of oscillator coupling. Generalizing this idea, a single master cyclin-CDK oscillator could entrain independent oscillators controlling multiple cell cycle events (e.g., Cdc14 release, periodic transcription, centrosome duplication, and budding) (Figure 3B–C). If this were the case, then decreasing the amplitude of cyclin-CDK oscillations should weaken the entrainment of peripheral oscillators, leading to disorder of the controlled events (due to different intrinsic frequencies of the oscillators). Indeed, reducing the amplitude of cyclin-CDK oscillations in freely cycling cells led to alterations of timing and sporadic skips or extra executions of normally strictly ordered, once-per-cycle events[56••].

This phase-locking mechanism can account for results that otherwise seem contradictory. For example, periodic, once-per-cell-cycle transcription seemed well accounted for by known cyclin-CDK regulation of various transcription factors; this accounting appeared quite sufficient until the transcriptional cycle was reported to cycle autonomously, independently of the cyclin-CDK cycle[57]! The phase-locking perspective accounts for this discrepancy by proposing that the known regulatory links between cyclin-CDK and transcription factors constitute coupling mechanisms between the two cycles, rather than constituting the oscillatory mechanism itself. The intrinsic oscillatory mechanism could be the sequential activation of transcription factors required for the next wave[59]; coupling could come about by additional regulation of these factors by the cyclin-CDK cycle[60]. The negative feedback oscillator proposed to regulate Cdc14 activity is similarly entrained to the cyclin-CDK cycle at multiple points [56••].

Before phase locking can be proposed as a generally satisfactory quantitative solution, measurements of the strength and timing of oscillator coupling between cyclin-CDK and multiple independent peripheral oscillators must be made. Experiments such as those carried out for Cdc14 release give enough quantitative restraints to make an initial positive statement, and a variant of this approach should be usable in other contexts.

While we have described the non-CDK oscillators as ‘peripheral,’ all of them impinge directly or indirectly on the cyclin-CDK oscillator itself. Such two-way communication almost surely tightens oscillator coordination, and could also account for robust cycling in the absence of checkpoints, or following ablation of transcriptional controls (as a striking example, the entire G1/S transcriptional program can be made constitutive at a low level without preventing viability, provided one G1 cyclin is constitutively expressed)[61].

Concluding Remarks

In this review, we have examined the intrinsic features of the cell cycle oscillatory circuit that control frequency (positive feedback loops, checkpoint control), the coupling of the frequency to external oscillators (metabolic cycle, circadian clock), and the coupling of internal oscillators to the cyclin-CDK cycle in order to coherently regulate cell cycle events. This complex regulation may provide several advantages. One is robustness, both through the enhancement of coherence discussed above and through simple redundancy of regulatory circuits[8]. The second may be flexibility. Positive feedback can allow tunable frequencies, which can promote evolvability[11•]. The cell cycle oscillator may be yoked to other clocks, such as the circadian clock, to increase fitness through coordination of processes. The

different gate points between the two cycles in different cell types suggests that this coupling may have been subjected to different selection pressures in different lineages. Similarly, peripheral cell cycle oscillators may have evolved independently to promote cyclic events in primitive cells, then been yoked to a later-evolving CDK cycle for a fitness advantage[56••].

Coupled oscillators, both within the cell cycle and between the cell cycle and external clocks, seem to be an increasingly common mode of cell cycle regulation. Phase-locking is a theme that covers the entire range of oscillator coordination, which may play a central role in determining oscillator frequency. Positive feedback, in turn, has been shown to be a frequent and perhaps essential mechanism of oscillator stabilization across a range of frequencies.

REFERENCES

1. Tyson JJ, Chen KC, Novak B. Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell. Curr Opin Cell Biol. 2003; 15:221–231. [PubMed: 12648679]
2. Novak B, Tyson JJ. Design principles of biochemical oscillators. Nat Rev Mol Cell Biol. 2008; 9:981–991. [PubMed: 18971947]
3. Felix MA, Labbe JC, Doree M, Hunt T, Karsenti E. Triggering of cyclin degradation in interphase extracts of amphibian eggs by cdc2 kinase. Nature. 1990; 346:379–382. [PubMed: 2142754]
4. Markson JS, O'Shea EK. The molecular clockwork of a protein-based circadian oscillator. FEBS Lett. 2009; 583:3938–3947. [PubMed: 19913541]
5. Dunlap JC, Loros JJ, Colot HV, Mehra A, Belden WJ, Shi M, Hong CI, Larrondo LF, Baker CL, Chen CH, et al. A circadian clock in Neurospora: how genes and proteins cooperate to produce a sustained, entrainable, and compensated biological oscillator with a period of about a day. Cold Spring Harb Symp Quant Biol. 2007; 72:57–68. [PubMed: 18522516]
6. King DP, Takahashi JS. Molecular genetics of circadian rhythms in mammals. Annu Rev Neurosci. 2000; 23:713–742. [PubMed: 10845079]
7. Elowitz MB, Leibler S. A synthetic oscillatory network of transcriptional regulators. Nature. 2000; 403:335–338. [PubMed: 10659856]
8. Cross FR. Two Redundant Oscillatory Mechanisms in the Yeast Cell Cycle. Developmental Cell. 2003; 4:741–752. [PubMed: 12737808]
9. Sha W, Moore J, Chen K, Lassaletta AD, Yi C-S, Tyson JJ, Sible JC. Hysteresis drives cell-cycle transitions in Xenopus laevis egg extracts. PNAS. 2003; 100:975–980. [PubMed: 12509509]
10. Pomerening JR, Sontag ED, Ferrell JE. Building a cell cycle oscillator: hysteresis and bistability in the activation of Cdc2. Nat Cell Biol. 2003; 5:346–351. [PubMed: 12629549]
11. Tsai TY, Choi YS, Ma W, Pomerening JR, Tang C, Ferrell JE. Robust, tunable biological oscillations from interlinked positive and negative feedback loops. Science. 2008; 321:126–129. [PubMed: 18599789] . In this theoretical work, the authors test various oscillator models to compare negative-feedback-only and negative-plus-positive-feedback architectures. They demonstrate that the addition of positive feedback to an oscillator provides tunability, allowing the system to operate over a broad range of frequencies without diminished amplitude.
12. Morgan, DO. The Cell Cycle: principles of control. edn 1. London: New Science Press, Ltd.; Oxford University Press; Sinauer Associates, Inc.; 2007.
13. Hartwell LH, Weinert TA. Checkpoints: controls that ensure the order of cell cycle events. Science. 1989; 246:629–634. [PubMed: 2683079]
14. Bean JM, Siggia ED, Cross FR. Coherence and Timing of Cell Cycle Start Examined at Single-Cell Resolution. Molecular Cell. 2006; 21:3–14. [PubMed: 16387649]
15. Di Talia S, Skotheim JM, Bean JM, Siggia ED, Cross FR. The effects of molecular noise and size control on variability in the budding yeast cell cycle. Nature. 2007; 448:947–951. [PubMed: 17713537]
16. Wang H, Carey LB, Cai Y, Wijnen H, Futcher B. Recruitment of Cln3 cyclin to promoters controls cell cycle entry via histone deacetylase and other targets. PLoS Biol. 2009; 7:e1000189. [PubMed:

19823669] . This work provides evidence for a cell size sensing mechanism based on titration of Cln3 molecules by SCB sites in promoters. The authors show that increasing SCB site number increases cell size at Start in a Cln3-dependent manner.

17. Di Talia S, Wang H, Skotheim JM, Rosebrock AP, Futcher B, Cross FR. Daughter-specific transcription factors regulate cell size control in budding yeast. PLoS Biol. 2009; 7 e1000221. . This paper describes the mechanistic basis for differential size control in mother and daughter cells in budding yeast. Mother cells retain all their mass from previous cell cycles; accordingly, they exhibit a pulse of *CLN3* expression upon division which may allow them to "skip" the next round of size control. Daughter cells, which lack this pulse, are subject to tight size control.
18. Laabs TL, Markwadt DD, Slattery MG, Newcomb LL, Stillman DJ, Heideman W. ACE2 is required for daughter cell-specific G1 delay in Saccharomyces cerevisiae. PNAS. 2003; 100:10275–10280. [PubMed: 12937340]
19. Charvin G, Oikonomou C, Siggia ED, Cross FR. Origin of irreversibility of cell cycle start in budding yeast. PLoS Biol. 2010; 8:e1000284. [PubMed: 20087409] . These two papers (see also Skotheim *et al.* (2008) below) describe a positive feedback loop which drives coherence and irreversibility of the Start transition, ensuring one-way entry into the cell cycle.
20. Skotheim JM, Di Talia S, Siggia ED, Cross FR. Positive feedback of G1 cyclins ensures coherent cell cycle entry. Nature. 2008; 454:291–296. [PubMed: 18633409] See Charvin *et al.* (2010) above.
21. Holt LJ, Krutchinsky AN, Morgan DO. Positive feedback sharpens the anaphase switch. Nature. 2008; 454:353–357. [PubMed: 18552837] . This work describes how positive feedback in the phosphoregulation of securin leads to coherent separation of sister chromatids at anaphase.
22. Martin SG, Berthelot-Grosjean M. Polar gradients of the DYRK-family kinase Pom1 couple cell length with the cell cycle. Nature. 2009; 459:852–856. [PubMed: 19474792] . These two papers (see also Moseley *et al.* (2009) below) address the mechanism of size control in *S. pombe*. They describe the polar localization of Pom1, an indirect inhibitor of mitotic CDK activity, and propose a model whereby increasing cell length decreases the Pom1 concentration in the middle of the cell, allowing mitosis above a minimal cell length.
23. Moseley JB, Mayeux A, Paoletti A, Nurse P. A spatial gradient coordinates cell size and mitotic entry in fission yeast. Nature. 2009; 459:857–860. [PubMed: 19474789] . See Martin and Berthelot-Grosjean (2009) above.
24. Sveiczer A, Novak B, Mitchison JM. The size control of fission yeast revisited. J Cell Sci. 1996; 109:2947–2957. [PubMed: 9013342]
25. Harvey SL, Kellogg DR. Conservation of Mechanisms Controlling Entry into Mitosis: Budding Yeast Wee1 Delays Entry into Mitosis and Is Required for Cell Size Control. Current Biology. 2003; 13:264–275. [PubMed: 12593792]
26. McNulty JJ, Lew DJ. Swe1p Responds to Cytoskeletal Perturbation, Not Bud Size, in S. cerevisiae. Current Biology. 2005; 15:2190–2198. [PubMed: 16360682]
27. Lee KS, Asano S, Park JE, Sakchaisri K, Erikson RL. Monitoring the cell cycle by multi-kinase-dependent regulation of Swe1/Wee1 in budding yeast. Cell Cycle. 2005; 4:1346–1349. [PubMed: 16123596]
28. Bramkamp M, Emmins R, Weston L, Donovan C, Daniel RA, Errington J. A novel component of the division-site selection system of Bacillus subtilis and a new mode of action for the division inhibitor MinCD. Mol Microbiol. 2008; 70:1556–1569. [PubMed: 19019154]
29. Patrick JE, Kearns DB. MinJ (YvjD) is a topological determinant of cell division in Bacillus subtilis. Mol Microbiol. 2008; 70:1166–1179. [PubMed: 18976281]
30. Bowman GR, Comolli LR, Zhu J, Eckart M, Koenig M, Downing KH, Moerner WE, Earnest T, Shapiro L. A polymeric protein anchors the chromosomal origin/ParB complex at a bacterial cell pole. Cell. 2008; 134:945–955. [PubMed: 18805088]
31. Shapiro L, McAdams HH, Losick R. Why and how bacteria localize proteins. Science. 2009; 326:1225–1228. [PubMed: 19965466]
32. Biondi EG, Reisinger SJ, Skerker JM, Arif M, Perchuk BS, Ryan KR, Laub MT. Regulation of the bacterial cell cycle by an integrated genetic circuit. Nature. 2006; 444:899–904. [PubMed: 17136100]

33. Chen YE, Tsokos CG, Biondi EG, Perchuk BS, Laub MT. Dynamics of two phosphorelays controlling cell cycle progression in Caulobacter crescentus. J Bacteriol. 2009; 191:7417–7429. [PubMed: 19783630] . This work reveals the dual kinase/phosphatase activity of CckA driving two phosphorelays which alternately activate and inactivate the master regulator CtrA.
34. Iniesta AA, Hillson NJ, Shapiro L. Cell pole-specific activation of a critical bacterial cell cycle kinase. PNAS. 2010; 107:7012–7017. [PubMed: 20351295] . This paper describes recruitment of the essential CckA histidine kinase to the new cell pole following initiation of DNA replication, leading to kinase activation and downstream activity. This mechanism confers temporal order on events and ensures once-per-cell-cycle DNA replication.
35. Richard P. The rhythm of yeast. FEMS Microbiol. Rev. 2003; 27:547–557. [PubMed: 14550945]
36. Silverman SJ, Petti AA, Slavov N, Parsons L, Briehof R, Thiberge SY, Zenklusen D, Gandhi SJ, Larson DR, Singer RH, et al. Metabolic cycling in single yeast cells from unsynchronized steady-state populations limited on glucose or phosphate. PNAS. 2010; 107:6946–6951. [PubMed: 20335538]
37. Klevecz RR, Bolen J, Forrest G, Murray DB. A genomewide oscillation in transcription gates DNA replication and cell cycle. PNAS. 2004; 101:1200–1205. [PubMed: 14734811]
38. Tu BP, Kudlicki A, Rowicka M, McKnight SL. Logic of the yeast metabolic cycle: temporal compartmentalization of cellular processes. Science. 2005; 10:1152–1158. [PubMed: 16254148]
39. Chen Z, Odstrcil EA, Tu BP, McKnight SL. Restriction of DNA replication to the reductive phase of the metabolic cycle protects genome integrity. Science. 2007; 316:1916–1919. [PubMed: 17600220]
40. Bjarnason GA, Jordan RC, Wood PA, Li Q, Lincoln DW, Sothern RB, Hrushesky WJ, Ben-David Y. Circadian expression of clock genes in human oral mucosa and skin: association with specific cell-cycle phases. Am J Pathol. 2001; 158:1793–1801. [PubMed: 11337377]
41. Bjarnason GA, Jordan R. Rhythms in human gastrointestinal mucosa and skin. Chronobiol Int. 2002; 19:129–140. [PubMed: 11962671]
42. Smaaland R, Sothern RB, Laerum OD, Abrahamsen JF. Rhythms in human bone marrow and blood cells. Chronobiol Int. 2002; 19:101–127. [PubMed: 11962670]
43. Dekens MP, Santoriello C, Vallone D, Grassi G, Whitmore D, Foulkes NS. Light regulates the cell cycle in zebrafish. Curr Biol. 2003; 13:2051–2057. [PubMed: 14653994]
44. Matsuo T, Yamaguchi S, Mitsui S, Emi A, Shimoda F, Okamura H. Control mechanism of the circadian clock for timing of cell division in vivo. Science. 2003; 302:255–259. [PubMed: 12934012]
45. Nagoshi E, Saini C, Bauer C, Laroche T, Naef F, Shibler U. Circadian gene expression in individual fibroblasts: cell-autonomous and self-sustained oscillators pass time to daughter cells. Cell. 2004; 119:693–705. [PubMed: 15550250]
46. Yang Q, Pando BF, Dong G, Golden SS, van Oudenaarden A. Circadian gating of the cell cycle revealed in single cyanobacterial cells. Science. 2010; 327:1522–1526. [PubMed: 20299597]
47. Dong G, Yang Q, Wang Q, Kim YI, Wood TL, Osteryoung KW, van Oudenaarden A, Golden SS. Elevated ATPase activity of KaiC applies a circadian checkpoint on cell division in *Synechococcus elongatus*. Cell. 2010; 140:529–539. [PubMed: 20178745]
48. Drapkin BJ, Lu Y, Procko AL, Timney BL, Cross FR. Analysis of the mitotic exit control system using locked levels of stable mitotic cyclin. Mol. Syst. Biol. 2009; 5
49. Sluder G, Lewis K. Relationship between nuclear DNA synthesis and centrosome reproduction in sea urchin eggs. J Exp Zool. 1987; 244:89–100. [PubMed: 3694143]
50. Sluder G, Miller FJ, Cole R, Rieder CL. Protein synthesis and the cell cycle: centrosome reproduction in sea urchin eggs is not under translational control. J Cell Biol. 1990; 110:2025–2032. [PubMed: 2351692]
51. Gard DL, Hafezi S, Zhang T, Doxsey SJ. Centrosome duplication continues in cycloheximide-treated *Xenopus* blastulae in the absence of a detectable cell cycle. J Cell Biol. 1990; 110:2033–2042. [PubMed: 2190990]
52. Haase SB, Winey M, Reed SI. Multi-step control of spindle pole body duplication by cyclin-dependent kinase. Nat Cell Biol. 2001; vol 3:38–42. Edited by. [PubMed: 11146624]

53. Durcan TM, Halpin ES, Casaletti L, Vaughan KT, Pierson MR, Woods S, Hinchliffe EH. Centrosome duplication proceeds during mimosine-induced G1 cell cycle arrest. J Cell Physiol. 2008; 215:182–191. [PubMed: 17960592]

54. McCleland ML, O'Farrell PH. RNAi of mitotic cyclins in *Drosophila* uncouples the nuclear and centrosome cycle. Curr Biol. 2008; 18:245–254. [PubMed: 18291653] . This work shows continued cycling of centrosome duplication upon knockdown of mitotic cyclins in *Drosophila* embryos, suggesting an independent cycle that is normally entrained by mitotic cyclin-CDK activity.

55. Haase SB, Reed SI. Evidence that a free-running oscillator drives G1 events in the budding yeast cell cycle. Nature. 1999; 401:394–397. [PubMed: 10517640]

56. Lu Y, Cross FR. Periodic cyclin-Cdk activity entrains an autonomous Cdc14 release oscillator. Cell. 2010; 141:268–279. [PubMed: 20403323] This work describes a novel peripheral cell cycle oscillator in *S. cerevisiae* which controls the periodic release from nucleolar sequestration of the mitotic Cdc14 phosphatase. The authors integrate their observations with other reports of peripheral oscillators to propose a phase-locking model in which the frequencies of oscillators controlling cell cycle events are modulated by a master CDK oscillator to ensure once-per-cell cycle execution of those events.

57. Orlando DA, Lin CY, Bernard A, Wang JY, Socolar JES, Iversen ES, Hartemink AJ, Haase SB. Global control of cell-cycle transcription by coupled CDK and network oscillators. Nature. 2008; 453:944–947. [PubMed: 18463633] . The authors describe an intrinsic oscillatory network controlling transcription of *S. cerevisiae* cell cycle genes in the absence of CDK cycle progression (accomplished through deletion of S - and M-phase cyclins). It is a striking example of a fundamental non-CDK oscillator controlling essential cell cycle events.

58. Thornton BR, Toczyski DP. Securin and B-cyclin/CDK are the only essential targets of the APC. Nat Cell Biol. 2003; 5:1090–1094. [PubMed: 14634663]

59. Lee TI, Rinaldi NJ, Robert F, Odom DT, Bar-Joseph Z, Gerber GK, Hannett NM, Harbison CT, Thompson CM, Simon I, et al. Transcriptional regulatory networks in Saccharomyces cerevisiae. Science. 2002; 298:799–804. [PubMed: 12399584]

60. Wittenberg C, Reed SI. Cell cycle-dependent transcription in yeast: promoters, transcription factors, and transcriptomes. Oncogene. 2005; 24:2746–2755. [PubMed: 15838511]

61. Koch C, Moll T, Neuberg M, Ahorn H, Nasmyth K. A role for the transcription factors Mbp1 and Swi4 in progression from G1 to S phase. Science. 1993; 261:1551–1557. [PubMed: 8372350]

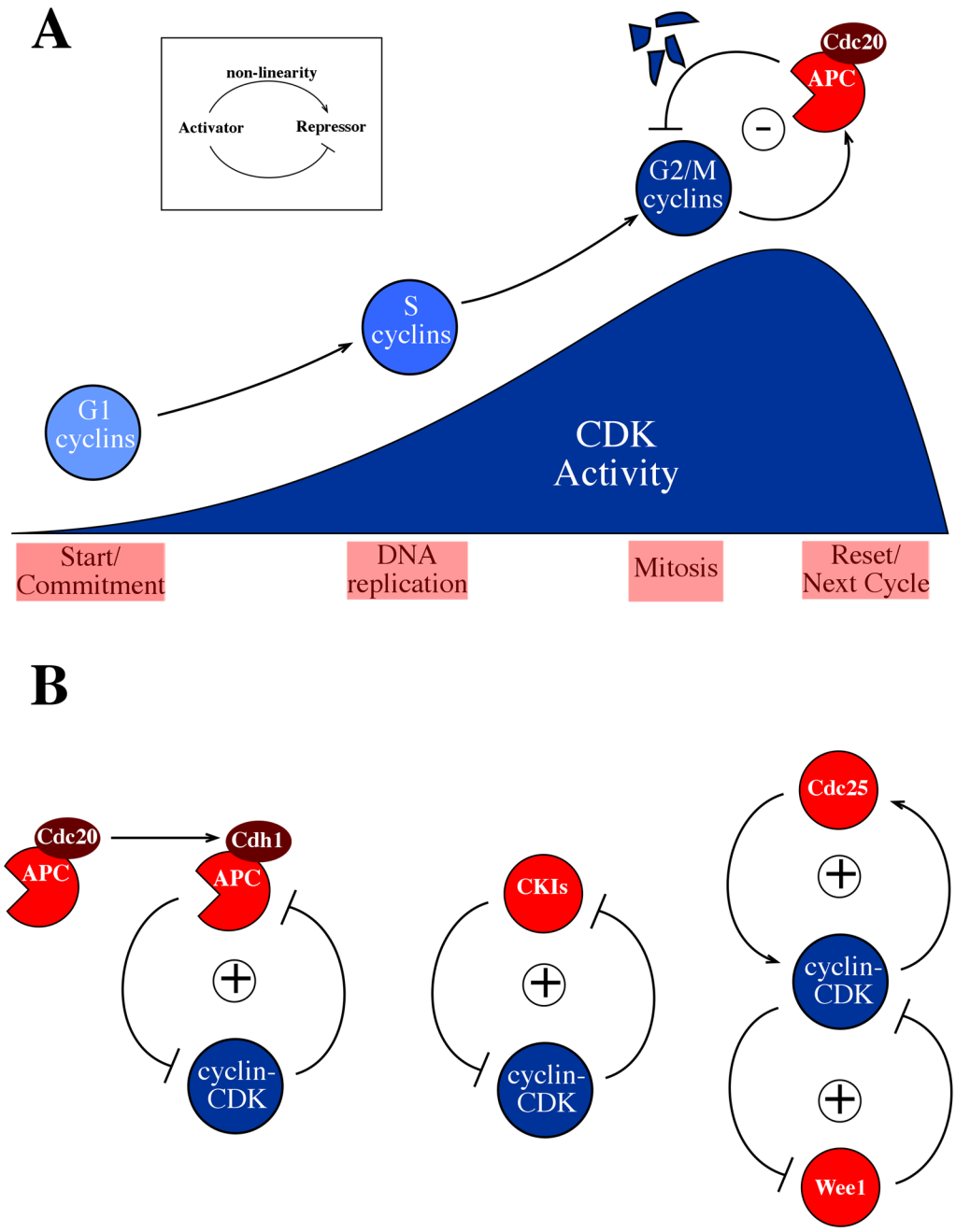

Figure 1.

Positive and negative feedback loops in the cyclin-CDK oscillator. **A** Inset: a negative feedback loop which can give rise to oscillations. Such an oscillator is thought to form the core of eukaryotic cell cycles, with cyclin-Cyclin Dependent Kinase (cyclin-CDK) acting as activator, Anaphase Promoting Complex-Cdc20 (APC-Cdc20) acting as repressor, and non-linearity in APC-Cdc20 activation preventing the system from settling into a steady state. Below is shown the cyclin-CDK machinery in eukaryotic cell cycles. CDKs, present throughout the cell cycle, require the binding of a cyclin subunit for activity. These cyclin partners can also determine the localization of the complex and its specificity for targets. At

the beginning of the cell cycle, cyclin-CDK activity is low, and ramps up over most of the cycle. Early cyclins trigger production of later cyclins and these later cyclins then turn off the earlier cyclins, so that control is passed from one set of cyclin-CDKs to the next. The last set of cyclins to be activated, the G2/M-phase cyclins, initiate mitosis, and also initiate their own destruction by activating the APC-Cdc20 negative feedback loop. APC-Cdc20 targets the G2/M-phase cyclins for destruction, resetting the cell to a low-CDK activity state, ready for the next cycle. **B** Positive feedback is added to the oscillator in multiple ways. Left: a highly-conserved but non-essential mechanism consists of “handoff” of cyclin proteolysis from APC-Cdc20 to APC-Cdh1. Cdh1 is a relative of Cdc20 which activates the APC late in mitosis and into the ensuing G1. Cdh1 is inhibited by cyclin-CDK activity, resulting in mutual inhibition (which is logically equivalent to positive feedback). Middle: antagonism between cyclin-CDK and stoichiometric CDK inhibitors (CKIs) results in positive feedback. These loops stabilize high- and low-CDK activity states. Right: a double positive feedback loop comprising CDK-mediated inhibition of the Wee1 kinase (which inhibits CDK) and activation of the Cdc25 phosphatase (which activates CDK by removing the phosphorylation added by Wee1) is proposed to stabilize intermediate CDK activity found in mid-cycle, and an alternative stable state of high mitotic CDK activity.

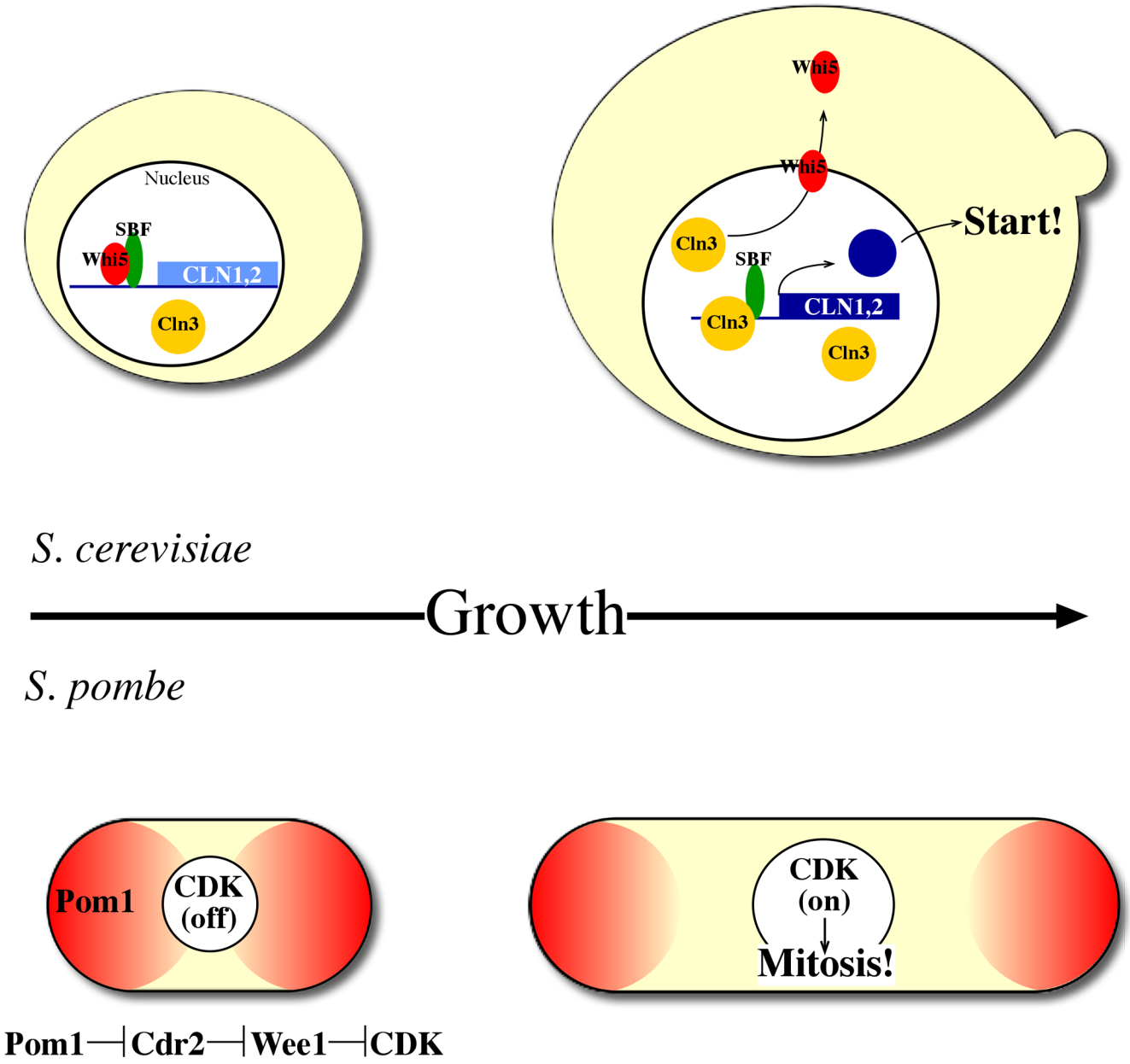

Figure 2.

Size control mechanisms in budding and fission yeasts. Top: in *S. cerevisiae*, size control operates in G1. Transcription of many genes, including the G1/S cyclins (Cln1,2) is controlled by the SBF and MBF transcription factors (Swi4/Swi6 and Swi4/Mbp1, respectively). The Whi5 repressor inhibits this transcription until it is exported from the nucleus by the most upstream G1 cyclin, Cln3, in response to sufficient cell size. Cln3 thus relieves transcriptional inhibition, promoting Cln1,2 expression and subsequent cell cycle Start. Actual size “measurement” was recently proposed to operate through direct binding of Cln3 to the SCB target sequences of Swi4/Swi6, with Start occurring upon titration of these sites by Cln3. Bottom: in *S. pombe*, size control operates in G2. Pom1, localized to cell poles, indirectly inhibits CDK activity (through inhibition of Cdr2, which inhibits Wee1,

which in turn inhibits CDK). As the cell elongates, the concentration of Pom1 at the center of the cell (where the nucleus is located) drops, allowing CDK activation leading to mitosis.

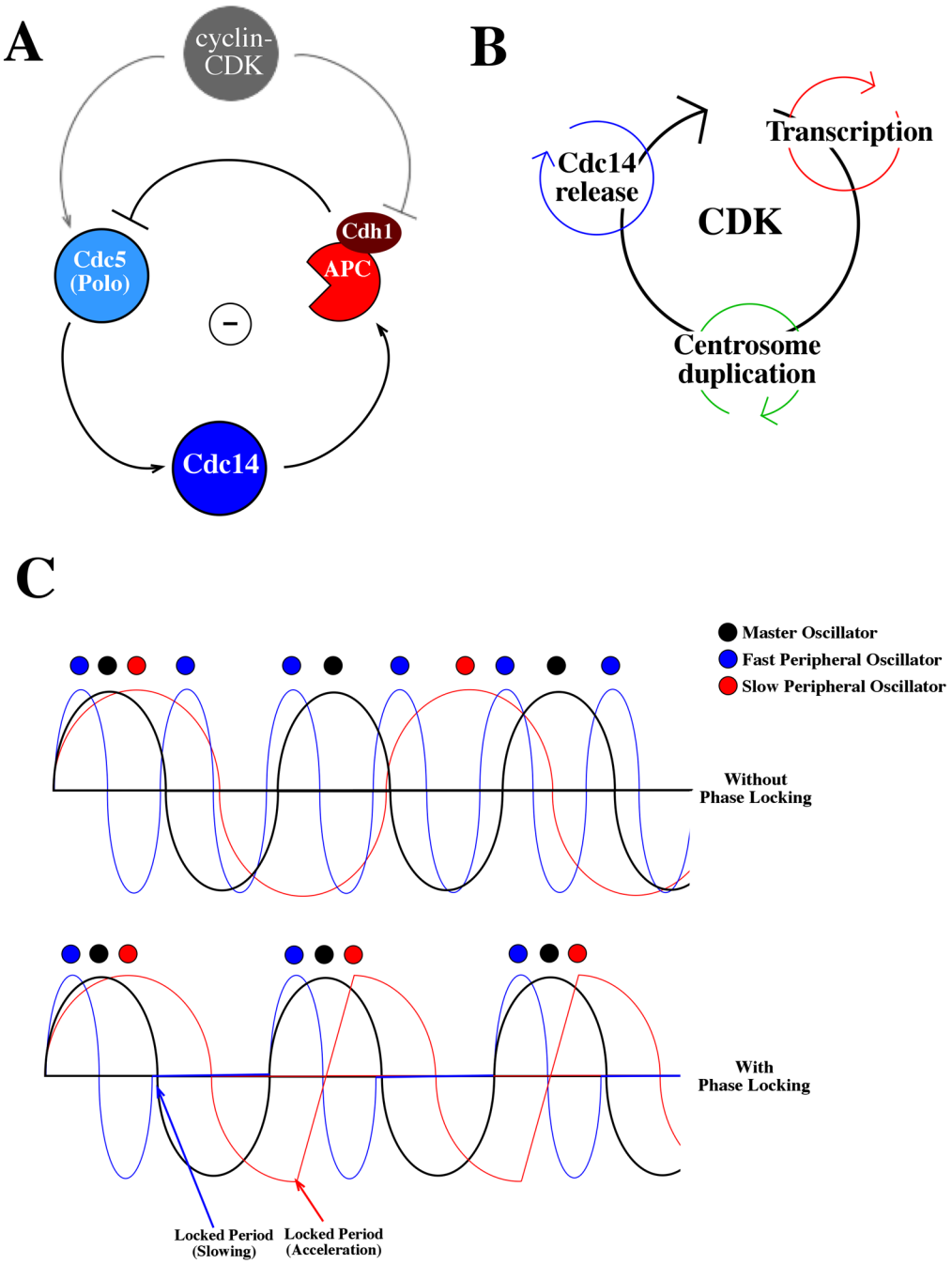

Figure 3.

A phase-locking model for entrainment of peripheral oscillators to the cyclin-CDK ocillator. **A** Molecular mechanism of the Cdc14 release oscillator. The mitotic phosphatase Cdc14 is activated upon release from sequestration in the nucleolus. This release is controlled by a negative feedback loop in which Cdc14 release, promoted by the polo kinase Cdc5, activates APC-Cdh1, which then promotes Cdc5 degradation, allowing Cdc14 resequestration. This negative feedback oscillator is entrained to the cyclin-CDK cycle at multiple points: by cyclin-CDK promotion of *CDC5* transcription and Cdc5 kinase activation, and by cyclin-CDK inhibition of Cdh1 activity. **B** Schematic of multiple peripheral oscillators coupled to

the CDK oscillator in budding yeast. As described above, coupling entrains such peripheral oscillators to cell cycle progression; peripheral oscillators also feed back on the cyclin-CDK oscillator itself. For example, major genes in the periodic transcription program include most cyclins, *CDC20*, and *CDC5*; Cdc14 directly promotes establishment of the low-cyclin-CDK positive feedback loop by activating Cdh1 and Sic1 as well as more indirectly antagonizing cyclin-CDK activity by dephosphorylating cyclin-CDK targets; the centrosome and budding cycles could communicate with the cyclin-CDK cycle via the spindle integrity and morphogenesis checkpoints. **C** Oscillator coupling ensures once-per-cell-cycle occurrence of events. Three hypothetical oscillators are shown: a master cycle in black, a faster peripheral cycle in blue, and a slower peripheral cycle in red. In the absence of phase-locking (top), the oscillators trigger events (colored circles) without a coherent phase relationship. In the presence of oscillator coupling (bottom), the peripheral oscillators are slowed or accelerated within their critical periods to produce a locked phase relationship, with events occurring once and only once within each master cycle.