Fifty years of cycling

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ABSTRACT **Fifty years ago, the first isolation of conditional budding yeast mutants that were defective in cell division was reported. Looking back, we now know that the analysis of these mutants revealed the molecular mechanisms and logic of the cell cycle, identified key regulatory enzymes that drive the cell cycle, elucidated structural components that underly essential cell cycle processes, and influenced our thinking about cancer and other diseases. Here, we briefly summarize what was concluded about the coordination of the cell cycle 50 years ago and how that relates to our current understanding of the molecular events that have since been elucidated.**

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The cell cycle is a process that orders a number of cellular processes to ensure the accurate duplication of the cell. It was hoped that a genetic analysis would reveal how the events were integrated. The inspiration for this was the work of Bob Edgar and Bill Wood on bacteriophage morphogenesis, which revealed the ordered steps by which phage parts were assembled and then put together (Wood and Edgar, 1967). The major questions were how DNA replication and spindle morphogenesis were integrated to achieve accurate chromosome segregation; how cell division was integrated with mitosis to ensure that both daughter cells received a full chromosome complement; and how growth and division were integrated to maintain a constant cell size.

Mutants that block cell cycle progression were identified by screening collections of randomly generated temperature sensitive mutants (Hartwell *et al.*, 1970a). Each mutant was screened individually by time-lapse photomicroscopy to identify cell division control (CDC) mutants that caused all cells in the population to arrest at the same point in the cell cycle at the restrictive temperature. The use of budding yeast was critical because the presence and size of the daughter bud provided a simple readout of where cells were in the cell cycle. The first collection of CDC mutants was derived from screening 1500 temperature-sensitive mutants and identified a total of 147 mutants, which fell into 32 complementation groups (Hartwell *et al.*, 1973). An example of one of the first mutants identified is shown in Figure 1. Wild-type cells are found at all stages of the cell cycle at the restrictive temperature (panel A), whereas the CDC mutant cells arrest in late in the cell cycle with large daughter buds (panel B).

The phenotypes of the mutants revealed some preliminary answers to the major questions (Hartwell *et al.*, 1970a,b). Assuming that the primary biochemical defect in a mutant was the process that stopped first, the following conclusions could be drawn. The elongation of the spindle was dependent on prior duplication of the spindle poles and the completion of DNA replication. Cell division and mitosis were coordinated because the formation of the daughter bud was dependent on spindle pole duplication in the previous cycle and cytokinesis was dependent on prior elongation of the spindle. Growth and division were coordinated because the *CDC28* (*CDK1)* function at Start required sufficient growth to initiate all the events of the cell cycle. Cell fusion during mating of haploid cells was coordinated with cell division because mating hormones arrested the cell cycle at the *CDC28* step and fusion was restricted to that step in the cell cycle.

These observations raised the question of how the dependence of events on one another was controlled. Two models were considered. One, named substrate–product, proposed that a late step was dependent on an early step because the latter was the substrate for the former (e.g., replicated DNA was a substrate for the spindle). The other was regulation, meaning either that signals from completion of an early event induced a late event or that an incomplete early event inhibited a late event. In one example, regulation was evident when a genetic analysis of how damaged DNA arrested nuclear division revealed a signaling pathway (the DNA damage

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Abbreviations used: CDC, cell division control; CDK1, cyclin dependent kinase 1; ssDNA, single stranded DNA.

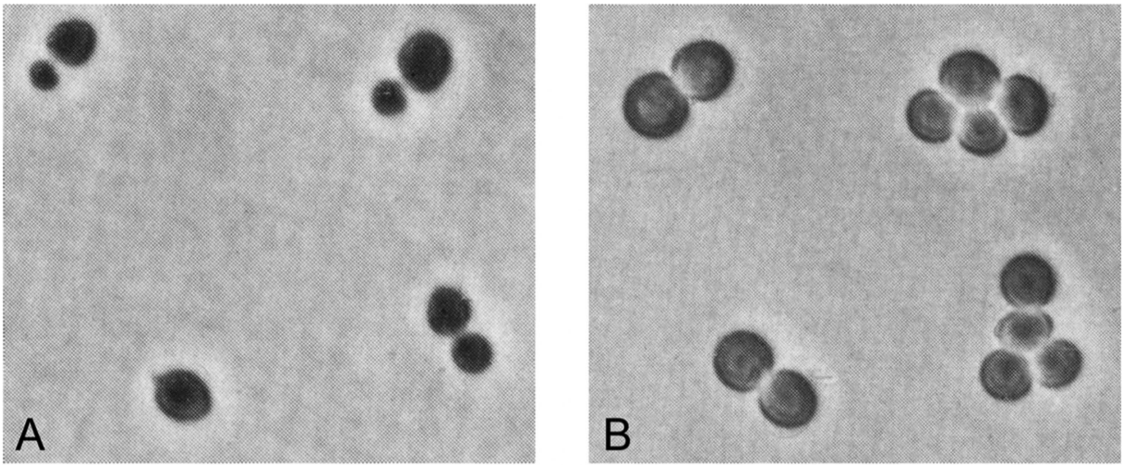

FIGURE 1: An example of one of the first CDC mutants isolated in budding yeast. Wild-type cells and temperature-sensitive mutant cells were grown at the permissive temperature and then shifted to the restrictive temperature, and CDCs were followed by photomicroscopy. (A) Wild-type cells, which are found at all stages of the cell cycle at the restrictive temperature, as indicated by the presence of cells at all stages of the daughter cell budding cycle. (B) A CDC mutant in which all cells have arrested at a cell cycle stage with large daughter buds.

checkpoint) that also accounted for why incomplete DNA replication prevented mitosis (Weinert and Hartwell, 1988).

The mechanisms underlying the dependence of cell cycle events upon one another have now been defined in considerable molecular detail. By way of illustration, we will briefly summarize what is known about the dependence of mitosis on replicated chromosomes and the dependence of cell division on mitosis. In some cases, the *cdc* mutants contributed to this work as a means to identify the relevant genes. However, in many cases, important components were not isolated as *cdc* mutants. Some of these have since been identified through biochemistry and subsequently shown to have Cdc phenotypes after mutants were created by in vitro mutagenesis. Additional cell cycle components were identified in other genetic screens or isolated using the original *cdc* mutants as starting points to search for genetic interactors. For example, the cyclins from yeast (which are redundant and nonessential), and even humans, were isolated, in part, as high-copy suppressors of the yeast *cdc28* mutant (Hadwiger *et al*., 1989).

One prominent class of *cdc* mutants affected DNA replication. Mutants targeting two of the three essential replicative polymerases were isolated, as were DNA ligase, a gene required for replication near telomeres, and genes required for the production of deoxyribonucleotides. We now know that these mutations resulted in robust arrest phenotypes because they led to the accumulation of significant amounts of ssDNA, the signal recognized by the replication checkpoint pathway (Zou and Elledge, 2003). This checkpoint signaling pathway both blocks mitosis and feeds back to replication. This feedback to replication helps stalled forks progress and also blocks origins that have not yet fired from doing so. The critical checkpoint phosphorylation events that effect these goals are well understood. Mitotic arrest is largely achieved by phosphorylation and stabilization of Pds1 (Cohen-Fix and Koshland, 1997), an event that blocks sister chromatid separation. Blocking origin firing is mediated by the phosphorylation of two proteins required for origin firing (Lopez-Mosqueda *et al*., 2010; Zegerman and Diffley, 2010). Finally, the restart of replication forks stalled by either mutational disruption or exogenous agents is promoted by the phosphorylation of several critical targets that increase nucleotide levels and modify the activity of proteins that act at the fork (Ciccia and Elledge, 2010). If measured by viability after fork arrest, this last function is by far the most significant role of this checkpoint pathway, although mitotic arrest and blocking origin firing are also important for preserving genome integrity.

While the *cdc* screen was effective in identifying genes involved in the mechanics of DNA replication, it was less effective in identifying genes that function exclusively to establish origins of replication. An exception to this, *CDC6*, sheds some light on why this may be. *cdc6* mutants brought to a fully nonpermissive temperature do not form replication forks, and thus do not activate the replication checkpoint, although they eventually arrest in mitosis due to the formation of an aberrant spindle that triggers the spindle assembly checkpoint (Piatti *et al*., 1995; Stern and Murray, 2001). Temperature-sensitive alleles of polymerase or ligases are likely to generate a few nonfunctional replication forks even when the alleles are weak, thus activating the checkpoint and providing a clear Cdc phenotype. Of course, it could not have been foreseen at the time of this screen that mutations in some cell cycle processes might eliminate the very signals for arrest that the screen was designed to identify.

Major progress has also been made in understanding the coordination of events needed to ensure successful chromosome segregation to daughter cells during mitosis. The spindle poles duplicate and separate to form a microtubule-based spindle. During DNA replication, the cohesin complex is loaded onto sister chromatids to keep them paired until the metaphase to anaphase transition. At the same time, the kinetochores that mediate attachment of chromosomes to the spindle microtubules assemble on centromeres. These are examples of substrate–product relationships where cohesin and kinetochores will assemble once the chromatin templates are available. Similarly, kinetochores make attachments to the spindle microtubules as soon as they are assembled. The spindle assembly checkpoint, a regulatory pathway, monitors kinetochore–microtubule interactions and halts the metaphase-to-anaphase transition until all chromosomes are properly attached (Hoyt *et al*., 1991; Li and Murray, 1991). Once the checkpoint is satisfied, cells activate the anaphase-promoting complex to release the linkage between sister chromatids and allow the spindle to elongate and pull chromosomes to opposite poles. As the spindle elongates into the daughter cell, the cell

reverses Cdc28 substrate phosphorylations to promote mitotic exit and cytokinesis.

How are all of these events coordinated? Surprisingly, although corresponding temperature-sensitive mutants exist for most mitotic genes, the *cdc* screen did not isolate the structural components of the yeast spindle, spindle pole, or kinetochore, with the exception of one pole mutant, *cdc31*. In contrast, the screen identified many signaling molecules that regulate the metaphase-to-anaphase transition. The *cdc* screen identified five subunits of the anaphase-promoting complex, which coordinates this transition by ensuring the degradation of proteins that lead to the removal of cohesion from chromosomes and the down-regulation of Cdc28 activity (King *et al.*, 1995; Sudakin *et al.*, 1995). One of the substrates that must be degraded is Pds1, the same protein that is the target of the DNA checkpoint (Cohen-Fix *et al.*, 1996). Pds1 inhibits the enzyme separase that releases cohesin to ensure the timely separation of sister chromatids (Ciosk *et al.*, 1998; Uhlmann *et al.*, 2000). The targeted degradation of Pds1 and cyclins by a single complex couples spindle elongation and chromosome segregation to Cdc28 inactivation. The spindle assembly checkpoint inhibits the anaphase-promoting complex, reinforcing the coordination between proper spindle attachment to chromosomes and anaphase progression (Hwang *et al.*, 1998; Kim *et al.*, 1998). After chromosome segregation, many Cdc28 substrates must also be dephosphorylated to exit from mitosis. Each of the essential kinases and phosphatases in this control system, called the mitotic exit network, were found in the *cdc* screen (Shou *et al.*, 1999; Visintin *et al.*, 1999). The mitotic exit network coordinates cytokinesis with the spindle delivering chromosomes to the daughter cell (Bardin *et al.*, 2000; Pereira *et al.*, 2000). Finally, several members of the septin ring that ensures cytokinesis, the last event in the cell cycle, were identified as *cdc* mutants. The septin mutants continue to bud, replicate DNA, and undergo mitosis in the next cell cycle, showing that completion of all events in the prior cell cycle is not necessarily required for progression. However, looking back, most of the key mitotic events are coordinated by regulatory events that reinforce the dependence of one event on the next, as opposed to the substrate–product relationship. Even in cases where there are clear substrate–product dependencies, such as spindle attachment to kinetochores, the cell has multiple regulatory mechanisms in place to halt the cell cycle until errors are detected and corrected, thus ensuring the proper execution of mitosis.

What do the next 50 years hold? The short examples above illustrate the tremendous progress that has been made in understanding the molecular mechanisms that ensure the coordination of cell cycle events. However, there are still major questions about its specificity, accuracy, and complexity, as well as how it is altered in disease. Specialized cell divisions such as meiosis and asymmetric cell division or modified cell cycle states such as quiescence require modifications to the cell cycle. The reconstitution of molecular events has helped to identify the minimal components and regulation required, but this has not accounted for the exquisite precision of these processes in the cell. The complexity of how individual cell cycle events integrate with other cellular processes such as metabolism is still in the early stages. Uncontrolled cell division is the root of cancer, so identifying therapeutic targets that specifically cause cancer vulnerabilities and avoid toxicity to normal cell divisions is still very much needed. In sum, many of the principles gained from cell cycle research have guided our thinking about biological processes; further elucidating the underlying mechanisms of the cell cycle will continue to influence fundamental biology and disease research for decades to come.

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