Fission yeast cell cycle mutants and the logic of eukaryotic cell cycle control

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ABSTRACT **Cell cycle mutants in the budding and fission yeasts have played critical roles in working out how the eukaryotic cell cycle operates and is controlled. The starting point was Lee Hartwell's 1970s landmark papers describing the first cell division cycle (CDC) mutants in budding yeast. These mutants were blocked at different cell cycle stages and so were unable to complete the cell cycle, thus defining genes necessary for successful cell division. Inspired by Hartwell's work, I isolated CDC mutants in the very distantly related fission yeast. This started a program of searches for mutants in fission yeast that revealed a range of phenotypes informative about eukaryotic cell cycle control. These included mutants defining genes that were rate-limiting for the onset of mitosis and of the S-phase, that were responsible for there being only one S-phase in each cell cycle, and that ensured that mitosis only took place when S-phase was properly completed. This is a brief account of the discovery of these mutants and how they led to the identification of cyclin-dependent kinases as core to these cell cycle controls.**

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Lee Hartwell's two papers on CDC mutants in budding yeast (Hartwell *et al*., 1970, 1973) were a turning point in my scientific career. I was a 23-year-old graduate student at the University of East Anglia in Norwich, England, struggling to get a highly temperamental prototype amino acid analyzer to work. This machine kept switching itself off when the pressure in its chromatography columns became too high, instantly stopping it and dumping the sample being analyzed and all the amino acid data that had been collected during that analysis. I only got it working by inactivating all the pressure safety devices with a variety of devices including rubber bungs, Blu Tack, and adhesive tape. However, this meant I had to sit with the machine for hours watching the pressure dial so that I could switch it off when the pressure really did go dangerously high. During those long hours, I read many papers, including Lee's two papers describing cell division cycle (CDC) mutants and the genetic control of cell division. I was already interested in the CDC, particularly in how it was controlled, because it is the simplest example of biological reproduction, a core characteristic of all life. It was something I wanted to study, but I did not know how to go about it, and Lee's papers changed all that by demonstrating how genes could be identified that were needed for the cell cycle. I decided that this was what I should do after my PhD, when I could abandon amino acid analyzers forever.

I decided not to work with budding yeast but with fission yeast because it divided by cell fission, more typical of eukaryotic cells. But to do this I first needed to learn the genetics of fission yeast. This I did by spending 6 months in Bern, Switzerland during 1973, generously hosted by Urs Leupold. Then I moved as a postdoc to Murdoch Mitchison's laboratory in Edinburgh, Scotland, where he worked on the fission yeast cell cycle. Lee had isolated his CDC mutants by monitoring bud size, but that was impossible for fission yeast, which does not divide by budding. Fission yeast is a filamentous rod, dividing by medial fission once it has grown to a specific cell size. Thinking about how CDC mutants could be found in fission yeast, I came across a paper from Brenner and Jacob, who isolated mutants in genes required for DNA replication in *Escherichia coli* that blocked cell division. These mutants led to the bacterial filamentous rods becoming highly elongated (Kohiyama *et al*., 1963). That was the approach I used in fission yeast, identifying temperature-sensitive mutants that could not divide and so became elongated. These early studies defined about 30 *cdc* genes necessary for the successful completion of the cell cycle (Nurse *et al*., 1976; Nasmyth and Nurse, 1981).

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Abbreviations used: CDC, cell division cycle; CDK, cyclin dependent kinase; cdt, cdc10 dependent transcript; DNA, deoxyribose nucleic acid; rum, replication uncoupled from mitosis.

This was a beginning, but were any of these genes involved in *controlling* the cell cycle rather than just being *needed* for the cell cycle? I reasoned that controls over the cell cycle were likely to operate at rate-limiting steps in the cell cycle progression. To identify rate-limiting cell cycle genes required mutants with a different phenotype. If mutants were altered in a rate-limiting step so that they were advanced through the cell cycle, then they would divide more rapidly than they could grow and would divide at a smaller size. These mutants would identify genes that were rate-limiting for overall cell cycle progression. I called these mutants of small dividing cells "wee," the Scottish word for small, because they were isolated in Edinburgh (Nurse, 1975; Nurse and Thuriaux, 1980). I have to admit, though, that the first wee mutant was isolated completely by accident. In fact, I was trying to isolate elongated cdc cell cycle mutants by spinning a population of mutagenized cells through a gradient and selecting large cells, but what I found was cells dividing at a small size. This wee mutant was probably at that position in the gradient because the wee cells had become clumped. Two *wee* genes were found, *wee1* and w*ee2*. When these were crossed to the 30 *cdc* mutants that had been isolated, it turned out that *wee2* was a mutant allele of *cdc2*. Temperature-sensitive mutants of *cdc2* blocked in G2 before mitosis and *wee* mutant alleles of *cdc2* were advanced into mitosis, indicating that *cdc2* encoded a protein that was rate-limiting for the onset of mitosis. When Cdc2 was inactivated, cells could not undergo mitosis, and when Cdc2 was overactive, it completed G2 more rapidly and cells underwent mitosis at a small size.

Further examination of the *cdc2* mutant phenotype revealed that Cdc2 was required not only for the onset of mitosis but also for the onset of S-phase (Nurse and Bissett, 1981). The *cdc2* mutant blocked at START the point of commitment to the cell cycle in G1, which Lee had defined from his studies of *CDC28* in the budding yeast. This really excited me, because it meant that in fission yeast a single gene, *cdc2*, acted at two major *control* points in the cell cycle, at the point of commitment in G1 at the beginning of the cell cycle, and then again in G2 toward the end of the cell cycle at the onset of mitosis and cell division. To investigate the possibility that there was a *cdc2* gene in budding yeast, my lab used a budding yeast gene library to determine if it contained any genes that could rescue a *cdc2* mutant in fission yeast. That experiment identified a budding yeast DNA segment that contained a gene that could rescue a *cdc2* mutant, a gene that turned out to be the budding yeast *CDC28* gene (Beach *et al*., 1982)! This was a piece of luck, because only four budding yeast cell cycle genes had been cloned at that time. This result meant that the logic of cell cycle control was conserved across the two yeasts, working through *CDC28* in budding yeast and *cdc2* in fission yeast, genes that encoded proteins that were very similar in sequence. A little later, my laboratory used the same cloning approach to see if a human gene existed that could also rescue a *cdc2* mutant (Lee and Nurse, 1987). Surprisingly that experiment also worked, and so it could be concluded that the basis of cell cycle control was the same in yeasts and humans, and therefore it was likely to be conserved in all eukaryotes.

This analysis was rather abstract, based on genetics and logic. But what did Cdc2 actually do? Its protein sequence indicated similarity to a viral protein kinase, so my laboratory tested whether Cdc2 was also a protein kinase. We did this by expressing Cdc2 in bacteria, preparing antibodies against the Cdc2 protein, and showing that it had protein kinase activity (Simanis and Nurse, 1986). This activity was regulated by tyrosine phosphorylation, which in turn was controlled by the Wee1 protein kinase and a Cdc25 phosphatase (Russell and Nurse, 1986, 1987; Gould and Nurse, 1989). These results led to the conclusion that a phosphorylation cascade regulates eukaryotic cell cycle progression, controlling Cdc2 protein kinase activity, and that this protein kinase is central to two main control points in the cell cycle, the first in G1 at the onset of S-phase and the second in G2 at the onset of mitosis.

Further analysis of cell cycle mutants in fission yeast revealed that the Cdc2 protein kinase and its activating Cdc13 cyclin partner, which together make up cyclin-dependent kinase (CDK), had yet further roles in controlling the cell cycle. Heat-shocking temperature-sensitive mutants in *cdc2* and *cdc13* resulted in cells becoming diploid. We reasoned that destroying CDK activity in G2 cells might induce another round of the S-phase, implicating CDK in ensuring that there is only one S-phase in each cell cycle (Broek *et al*., 1991). This possibility was confirmed when the lab showed that eliminating Cdc13 (Hayles *et al*., 1994) or over expressing the CDK inhibitor Rum1 resulted in cells undergoing repeated rounds of DNA replication (Moreno and Nurse, 1994). We explained this with a model in which low CDK activity was required to initiate the S-phase, and as cells progressed into G2, CDK activity increased and suppressed further rounds of DNA replication. By destroying CDK activity in G2, cells were reset back to G1 and underwent another round of DNA replication. Increasing CDK activity through the cell cycle therefore underpins the temporal order of S-phase and mitosis.

Looking for mutants that underwent extra rounds of DNA replication also provided a way to identify genes that are rate-limiting for the onset of the S-phase. Overexpression screens identified the DNA replication initiation factors Cdc18 (the paralogue of CDC6 in budding yeast and other eukaryotes) and Cdt1 (Nishitani and Nurse, 1995; Yanow *et al*., 2001). When the levels of these two proteins are increased, cells are forced into DNA replication, suggesting that they play key rate-limiting roles at the onset of the S-phase. Biochemical work from the lab of John Diffley established the molecular basis of these proteins in the control of DNA replication and in ensuring that there is only one S-phase in each cell cycle.

A further class of fission yeast cell cycle mutants first identified by Mitsuhiro Yanagida is the "cut" mutants. This "cut" phenotype occurs when cells enter mitosis inappropriately but cannot complete it, so the nucleus becomes cut by cell division. A *cdc18*-deletion mutant blocks in G1 before the S-phase but then continues through the cell cycle and enters mitosis, leading to the cut phenotype. This indicates that in the absence of Cdc18, cells cannot activate the checkpoint control that blocks the onset of mitosis when the S-phase has not taken place (Kelly *et al*., 1993). Cells preparing for the S-phase need the Cdc18 DNA replication factor, but if it is absent, cells "forget" where they are in the cell cycle and undergo mitosis.

The genetic analysis of the cell cycle in fission yeast started with the identification of 30 *cdc* genes. With the availability of new genomic approaches, it became possible to identify all the genes required to complete the cell cycle on a genomewide basis. To address this question, my laboratory organized a systematic deletion of all fission yeast genes, which were then screened for *cdc* mutant defects. A total of 4836 genes were deleted, 96% of the genes annotated at that time. A screen for cell cycle defects identified 513 *cdc* genes, and a second screen identified 18 *wee* genes (Navarro and Nurse, 2012; Hayles *et al*., 2013). Therefore, over 500 genes are required for the cell cycle, about 10% of the total number of genes in fission yeast. It is to be hoped that this collection of genes will be helpful for investigations of the cell in the years to come.

It is nearly half a century since I first started to work on cell cycle mutants. They have given me endless pleasure and intellectual satisfaction, and I thank them for that. I also want to thank the colleagues in my laboratory who worked on the papers described in

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