

In 2001 Leland Hartwell, Tim Hunt and Paul Nurse were awarded the Nobel Prize in Physiology or Medicine for their discoveries of key regulators of the cell cycle (see <http://nobelprize.org/medicine/laureates/2001>).

▲ Sir Paul Nurse receiving his Nobel Prize from His Majesty the King of Sweden at the Stockholm Concert Hall in 2001. Hans Mehlin / nobelprize.org

Nobel microbes define the art of cell division

Because cancer is a disease of cell proliferation that often results in cells with a varied chromosome complement, understanding the control of cell division is an important goal in cancer research. It offers significant opportunities to develop novel therapies and to make existing ones more effective.

The eukaryotic cell cycle

Unlike its prokaryotic counterpart, the principal functions of the eukaryotic cell division cycle, to first replicate then segregate the genome, are partitioned into discrete phases. Cell division is not an inevitable event, rather it is a 'conscious' step and many criteria such as cell size and nutrient status must be fulfilled before cells 'decide' to enter the cell division cycle. Once committed, cells pass through the first gap phase (G1), DNA synthesis (S) and a second gap phase (G2) before dividing in mitosis (M) (Fig. 1).

Microbes in cell-cycle research

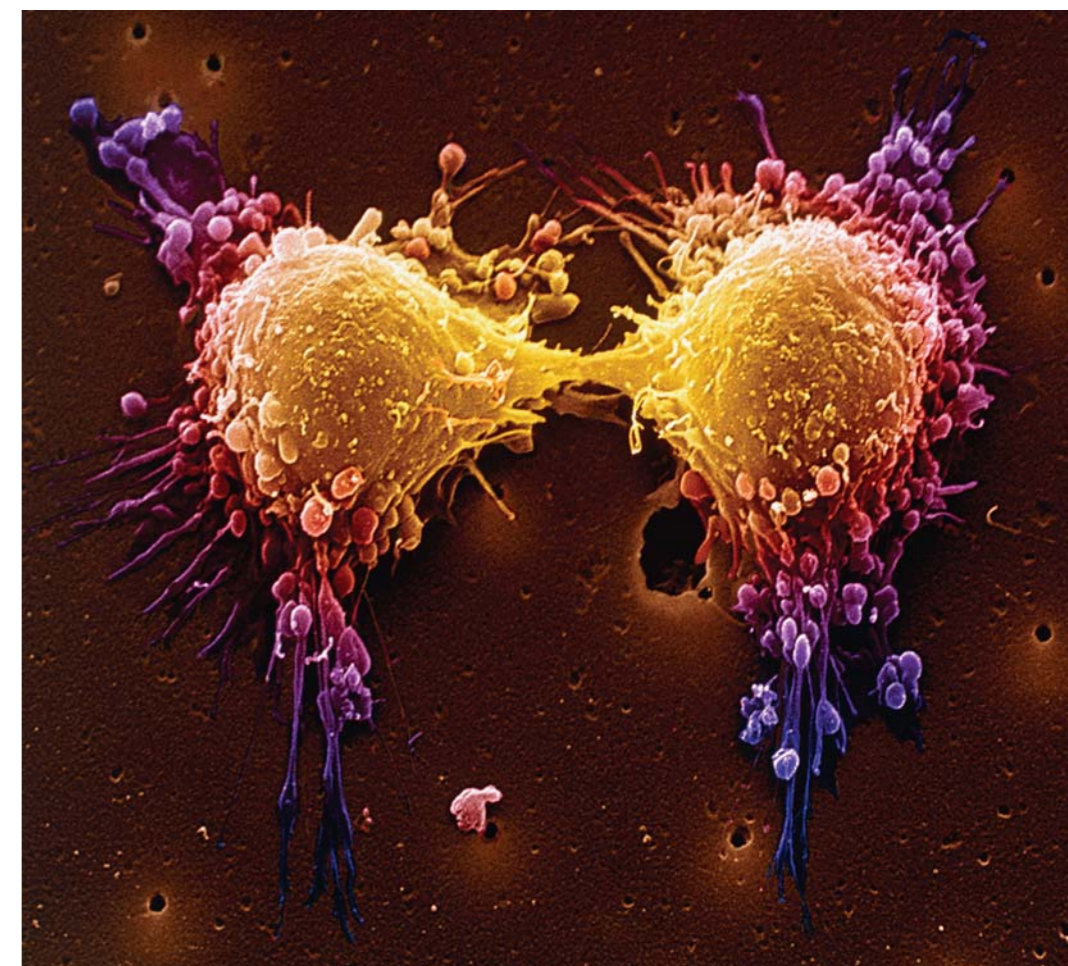
Several eukaryotic microbes have been instrumental in unlocking the secrets of the cell division cycle. Unlike the sophisticated metazoans, these model microbes, (*Tetrahymena*, *Chlamydomonas*, *Amoeba*, *Paramecium* and *Stentor*), have opted for a solitary lifestyle. As each generation in exponentially growing cultures is effectively a replica of the previous one, any variations in cell size or the time it takes to divide after particular manipulations reflect changes in cell-cycle control. Thus, the first convincing evidence for a requirement to attain a particular size prior to division came from studies of *Amoeba* by Prescott in 1956.

Physarum – the benefits of life as a giant pizza

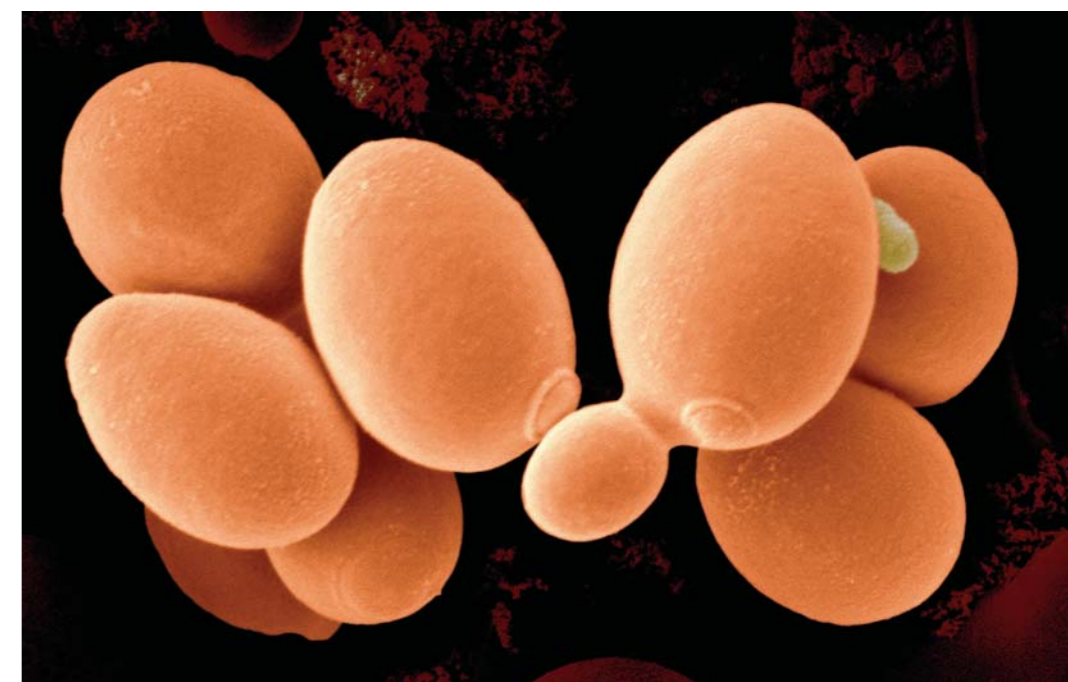
While the solitary microbes did their bit, the more complex slime mould *Physarum polycephalum* identified some core principles that became

In the fight against cancer, understanding the control of cell division is vital. **Iain Hagan** and **Paul Nurse** show the important role of micro-organisms in unlocking the secrets of the cell division cycle.

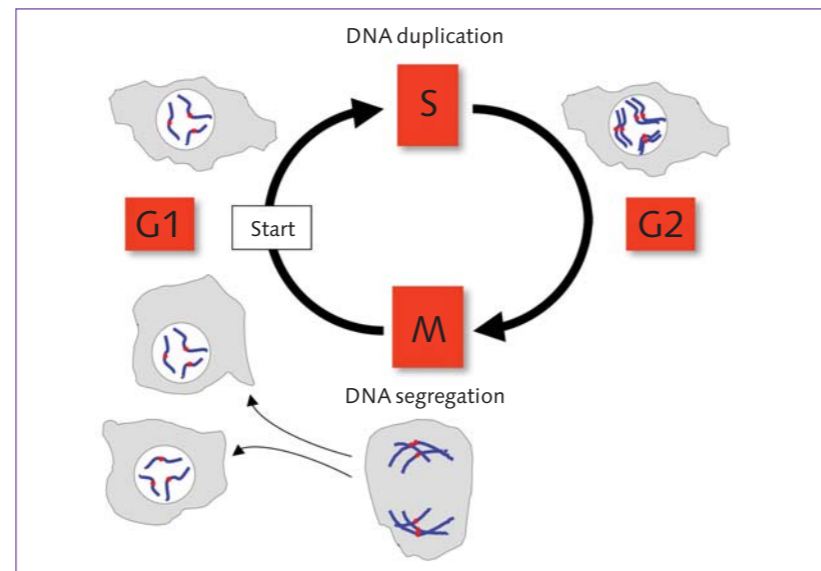
ingrained in cell-cycle lore after being repeated in higher eukaryotes. One of *Physarum*'s key attributes is its ability to live as a large body known as a syncytium in which thousands of functionally identical nuclei can synchronously duplicate and segregate their genomes in a common cytoplasm, enabling sufficient material to be gathered from a single cell to study the biochemical changes that accompany cycle progression. Its second critical attribute is the ability of syncytia to fuse. Thus, syncytia of different cell-cycle phases could be merged to elucidate how strict the controls are that govern the order of cell-cycle events. The inability of an S-phase syncytium to persuade G2 nuclei to undergo a second round of replication showed that DNA replication could not be solely governed by a soluble factor. In contrast, M-phase syncytia could push G2 nuclei into mitosis. The search for this soluble M-phase-promoting factor (MPF) took Bradbury and his colleagues remarkably close to uncovering the universal controls of cell division. For they found that bathing a G2 syncytium in a histone H1 kinase preparation whose activity peaked in mitosis, pushed G2 nuclei into mitosis.



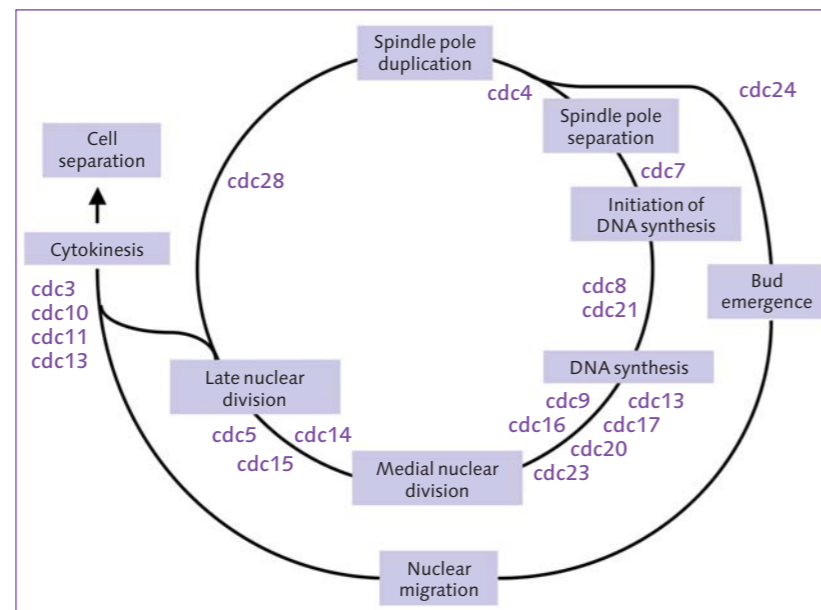
▲ Coloured scanning electron micrograph of two prostate cancer cells in the final stage of cell division (cytokinesis). During this stage the cells' cytoplasm divides. Here the cells are joined by a thin cytoplasmic bridge. Steve Gschmeissner / Science Photo Library



▲ Coloured scanning electron micrograph of cells of *Saccharomyces cerevisiae*. A daughter cell can be seen budding off one of the larger mother cells. Scimat / Science Photo Library



► Fig. 1. The eukaryotic cell division cycle. I. Hagan



► Fig. 2. Map of the budding yeast cell cycle. I. Hagan

The rise and rise of yeast

An emerging trend in the analysis of conserved biological problems is the eventual dominance of genetically malleable systems. The more efficient the genetics, the greater the impact of the system. Thus, analysis of mutants that displayed defects in cell-cycle progression in *Tetrahymena*, *Chlamydomonas* and *Aspergillus* has given way to the domination of the analysis of the budding yeast *Saccharomyces cerevisiae* and fission yeast *Schizosaccharomyces pombe*.

Budding yeast led the way

The seminal work of Leland Hartwell's laboratory in isolating a series of cell division cycle (*cdc*) mutants in the 1970s set the wheels in motion. Because genes that control cell-cycle progression will be essential for survival, he and his colleagues isolated a series of temperature-sensitive mutants that continued to grow but were unable to divide after the temperature of the culture was shifted from 25 to 37 °C. Again simple questions gave highly informative answers. The researchers reasoned that if a particular mutation results in an immediate arrest of cell division after the shift, then the number of cells that could divide after the shift would give a rough indication of where in the cell cycle that gene works. A lot of cell division

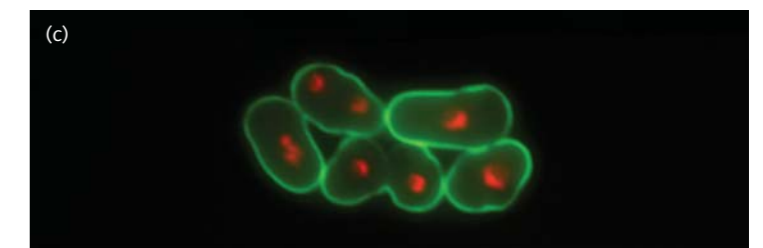
after the shift indicates an 'execution point' near the beginning of the cell cycle; little cell division indicates a function towards the end of the cycle. The next reciprocal shift experiments investigated whether cells went straight into division or needed to complete a cell-cycle event (e.g. S phase) after being released from execution points. The result was a map of the cell cycle in which particular genes acted at particular times (Fig. 2). Most importantly Hartwell postulated the existence of a commitment point in G1 called 'Start'. Before Start, cells could undergo the alternative fates of mating and sporulation but once past this point they had to go all the way through the cell cycle before the chance to mate would come again. As *cdc28* mutants arrested at the earliest phase of Start, Hartwell concluded that the product of the *cdc28+* gene was likely to play a critical role in cell-cycle control.

wee mutants give fission yeast an edge

Growth by linear extension and division by medial fission drew Mitchison to a seminal career in the study of the *Sch. pombe* cell cycle in the early 1950s. The rationale was simple; small cells were at the beginning of the cell cycle and large cells at the end. Therefore cell length not only gave an accurate measure of the cell-cycle status of a cell, but selecting small

It is clear that microbes have advanced our understanding of cell-cycle control by many decades

► Fig. 3. Fluorescence micrographs of wild-type (a), *cdc2.33* mutant (b) and *wee1.50* mutant (c) strains of *Sch. pombe*. I. Hagan



cells gave a culture that was synchronized with respect to cell-cycle progression. The application of genetics in the mid-1970s had a further impact on cell-cycle research as a novel type emerged from the isolation of cell-cycle mutants: the *wee* mutant.

wee mutations made cells shorter than normal because they speeded up the rate of cell-cycle progression (Fig. 3). Classical genetic dominance relationships drew particular attention to one of the two *wee* loci, *cdc2*. The two *cdc2* *wee* mutants were dominant and so represented a gain of function, while the loss of function mutants were classic *cdc* mutants. The ability of mutations in a single gene to accelerate or block cell-cycle progression put *cdc2* at the heart of cell-cycle control. Strikingly, *Sac. cerevisiae* *cdc28* could complement *Sch. pombe* *cdc2* mutants and *cdc2* acted at the two points that controlled the rate of progress through the fission yeast cell cycle: Start and the commitment to mitosis. Subsequent analysis showed that *cdc2* encoded a protein kinase and that the mitosis-promoting activity of this kinase was blocked by phosphorylation in the catalytic site by a protein kinase encoded by the *wee1* gene. Removal of the phosphate by the product of the *cdc25* gene promoted commitment to mitosis.

wee1/cdc25 control of cdc2.cyclin B regulates mitotic commitment in all eukaryotes

The true importance of the genetic approach in yeast was realized in a period of intense activity in the late 1980s. First, a human gene that could compensate for the loss of function in *Sch. pombe* *cdc2* was cloned and found to be a highly related gene – human *cdc2* (Fig. 4). Almost simultaneously the purification of a mitosis-promoting factor from frog eggs showed that it was composed of frog *cdc2* and a regulatory subunit called cyclin. Cyclins had become a focus of attention after Tim Hunt first showed that they were degraded once per cycle as cells divided. Furthermore, it emerged that the MPF was none other than the histone H1 kinase shown to promote mitosis in the *Physarum* syncytia 15 years previously. Thus, genetic approaches in yeast had identified the conserved means by which eukaryotes regulate commitment to mitosis. We now know that human cells possess *wee1-* and *cdc25*-related molecules, and a range of cyclin-dependent kinases (CDKs), and that these preside over all the rate-limiting steps in the human cell cycle. Molecules that play critical roles in carcinogenesis, directly or indirectly regulate these CDKs.

Budding yeast bites back – the concept of cell-cycle checkpoints

Lee Hartwell's studies of the budding yeast cell cycle were to lead him to one of the most influential contributions of the cell-cycle field to cancer research. Spurred on by numerous cases of dependency relationships, where a range of defects all arrested cell-cycle progression at similar points, Weinert and Hartwell proposed the existence of surveillance mechanisms that blocked cell division if previous cell-cycle events had not been completed or DNA integrity was compromised. They called these pathways cell-cycle checkpoints. Their elegant paper describing the DNA damage checkpoint prompted a huge body of work that makes it clear that one of the major distinctions between cancer cells and normal cells is that they are deficient in many of these check-point pathways.

Cell-cycle molecules and cancer therapy

Instead of having a reliable system of overlapping checkpoints many cancers are running on damaged or no checkpoints. This is probably why tumours are more sensitive to genotoxic drugs or ionizing radiation. The checkpoint pathways in normal cells arrest cell division in response to damage while the tumour cells continue dividing and so die. Thus not only are drugs that specifically target the checkpoint components in clinical trials at present, but ways are being sought to reinstate checkpoint controls to cancerous cells so that the abnormal genomes they possess trigger checkpoint pathways, resulting in cell-cycle arrest and eventually clearance through programmed cell death pathways.

Continuing apace

We halt our account in the early 1990s, but studies in yeast have continued to

tell us how cells target specific proteins for destruction at specific cell-cycle stages, how chromosome architecture is modulated and repaired and has even got to the point where fission yeast is arguably the best system to study the mechanism underlying the mainstay of the new form of higher eukaryotic genetics – RNAi. It is clear that microbes have advanced our understanding of cell-cycle control by many decades.

Professor Iain Hagan

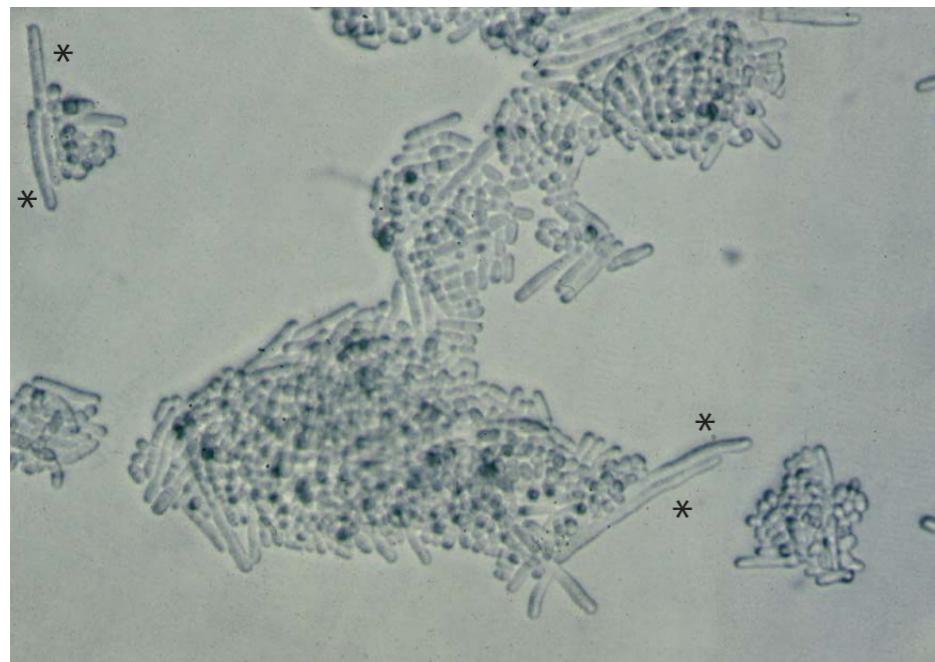
Paterson Institute for Cancer Research, Wilmslow Road, Manchester M20 4BX, UK
(t 0161 446 8193 / 446 3166; f 0161 446 3109; e IHagan@picr.man.ac.uk)

Professor Sir Paul Nurse

President, Rockefeller University, 1230 York Avenue, New York, NY 10021-6399, USA (t +1 212 327 8080; f +1 212 327 8900, e nurse@rockefeller.edu)

Further reading

- Berger, J.D.J. (2001).** Riding the ciliate cell cycle – a thirty five year perspective. *Euk Microbiol* **48**, 505–518.
- Hartwell, L.H. & Weinert, T.A. (1989).** Checkpoints: controls that ensure the order of cell cycle events. *Science* **246**, 629–634.
- Mitchison, J.M. (1996).** Cell cycles. In *Foundations of Modern Biochemistry. Quantum Leaps in Biochemistry, Vol. 2*, 203–230. Edited by M.G. Ord & L.A. Stocken. Greenwich, CT: JAI Press.
- Mitchison, J.M. (2003).** Growth during the cell division cycle. *Int Rev Cytol* **226**, 165–258.
- Nasmyth, K. (2001).** A prize for proliferation. *Cell* **107**, 689–701.
- Nasmyth K. (2002).** Segregating sister genomes: the molecular biology of chromosome separation. *Science* **297**, 559–565.
- Nurse, P. (1990).** Universal control mechanism regulating the onset of S phase. *Nature* **344**, 503–507.



▲ Fig. 4. Human *cdc2* complements temperature sensitive mutations in fission yeast *cdc2*. The image shows colonies of fission yeast *cdc2.33* mutants that are able to divide because they carry the human *cdc2* gene on a multi-copy plasmid. When they lose the plasmid they are unable to divide and so elongate (indicated by asterisks). Reproduced with permission from *Nature* **327**, 31–35.