On fission

The 1990 Marjory Stephenson Prize Lecture

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I would like to begin by thanking the Society for the opportunity to deliver this Marjory Stephenson Prize Lecture called 'On Fission'. The rather curious title reflects the two major interests of my career: the fission yeast *Schizosaccharomyces pombe*, and the cell division cycle which ends with cell fission. In this lecture I shall describe how fission yeast has been used to investigate the cell cycle and how these studies have led to the development of a model of cell cycle control which is relevant to all eukaryotes, including human cells. It will be a particular pleasure during the course of the lecture to acknowledge my past and present collaborators, who have made major contributions to this work.

Fission yeast has been known to humankind for over a century. The first illustration of this organism that I have found is in a book published in 1890 entitled 'Micro-organisms and Fermentation' written by the Carlsberg brewer Alfred Jorgensen. In the chapter 'Alcoholic Ferments', he describes a *Schizosaccharomyces* species isolated from currants, and clearly illustrates cells dividing by the characteristic medial fission which gives this yeast its common name. Isolates were also described from ferments used for the manufacture of rum in the West Indies and Java, and of millet beer in East Africa. The local name of this beer is pombe, from which the scientific name of fission yeast is derived. Proper scientific investigation began around 1950 with Urs Leupold working in Denmark and Switzerland. His early interests were mainly concerned with understanding the genetic characteristics of the mating-type system. Over the following decades the work of his laboratory in Berne established all the basic procedures that are still used for classical genetic analysis of fission yeast. Nearly 500 genes have now been identified, and around 250 of these have been assigned to one of the three chromosomes. The other founding father of fission yeast studies was Murdoch Mitchison, who worked in Scotland. His major interest was the cell cycle. In the mid 1950s he changed from working with sea urchin eggs to fission yeast because of the yeast's mode of growth by tip elongation, which made investigation of growth patterns during the

cell cycle more straightforward. This topic of research has remained his major interest until the present day.

My involvement with fission yeast began after finishing my PhD in 1973, with a six month visit to Urs Leupold's laboratory in Berne. The objective of this visit was to learn how to carry out fission yeast genetics with the intention of applying this knowledge to investigate the cell cycle in Murdoch Mitchison's laboratory in Edinburgh. The power of such an approach had already been shown by Lee Hartwell's genetic work on the cell cycle in budding yeast, which began in the early 1970s. Initially, temperature-sensitive cell cycle mutants were isolated which defined a total of 15 *cdc* genes. This collection was expanded to twice this number with the collaboration of Pierre Thuriaux and Kim Nasmyth, who were also working with Murdoch Mitchison at this time. Subsequent work from Mitsuhiro Yanagida's laboratory in Japan has extended the number of cell cycle genes in fission yeast to around 50. The mutants are blocked at various stages throughout the cycle, identifying gene products required for progress through the cell cycle. Investigation of mutants blocked at different stages established that a point of commitment to the cell cycle was located in G1, beyond which the cell could not undergo alternative developmental programmes such as conjugation until the cycle in progress was completed. This commitment point was called 'start' after the analogous control in budding yeast. Passage through start required the two gene functions encoded by *cdc2⁺* and *cdc10⁺*. After start the cell begins the sequence of events which leads to the onset of S-phase.

A second control point in the cell cycle acts at the end of G2 and leads to the onset of mitosis. This was originally revealed by the isolation of *wee* mutants, which were advanced into mitosis and cell division at a reduced cell size. These mutants were important because they allowed the identification of genes whose products played key roles in determining the cell cycle timing of mitosis. Two genes were originally identified, *wee1⁺* and *cdc2⁺*. The *cdc2⁺* gene was unique in being required twice during the cycle, first in G1 before S-phase and then in

Fig. 1. Major phases of the cell cycle and the times of p34^{cdc2} action.

G2 before mitosis, both being important cell cycle control points (Fig. 1). Support for the existence of the second control point in G2 came from further experiments carried out in collaboration with Peter Fantès. These involved shifting cultures between different media supporting altered growth rates, and established that the onset of mitosis required attainment of a critical cell size which was modulated by growth rate.

These classical genetic approaches are a useful way to identify controls operating during a process such as the cell cycle, but they did not allow a molecular investigation of the gene functions involved. Such an analysis required a DNA transformation system so that the genes of interest could be cloned from a gene bank by complementation of the appropriate mutant function. This was my major objective after setting up my own laboratory in the University of Sussex in 1980. A transformation system was established with the collaboration of David Beach, which rapidly led to the isolation of a number of fission yeast genes. Amongst the first to be cloned were the mating type region, *cdc2⁺*, and *suc1⁺*. Subsequently, Paul Russell cloned three further mitotic control genes, *cdc25⁺*, *wee1⁺* and *nim1⁺*, which acted together in a regulatory network determining the activation of *cdc2⁺*, and as a consequence the cell cycle timing of mitosis. The stage was now set for a molecular analysis of the mitotic control which was subsequently pursued in my laboratory both in ICRF London and also in Oxford.

The mitotic control consists of two pathways, an inhibitory one consisting of the two putative protein kinases *wee1⁺* and *nim1⁺*, and an activatory one including the gene product of *cdc25⁺* (see Fig. 2). These two pathways regulate a third protein kinase encoded by the *cdc2⁺* gene which brings about the onset of mitosis. Viesturs Simanis demonstrated biochemically that *cdc2⁺* encoded a protein kinase by raising antibodies against the gene product expressed in *Escherichia coli*, and against synthetic peptides corresponding to regions of

the predicted open reading frame of the gene. These antibodies immunoprecipitated a 34 kDa phosphoprotein from fission yeast extracts with protein kinase activity *in vitro*. Kinase activity was present in proliferating cells but was much reduced in cells withdrawn from the cell cycle after nutrient deprivation. When Sergio Moreno assayed kinase activity during a synchronous culture, he found that it peaked in level as cells underwent mitosis. This peak required the *cdc25⁺* gene product, suggesting that onset of mitosis was brought about by *cdc25⁺* activation of p34^{cdc2} protein kinase activity.

The molecular basis of p34^{cdc2} protein kinase activation at the onset of mitosis has been investigated by Kathy Gould. She established that changes in phosphorylation play an important role. As the p34^{cdc2} kinase became activated the protein was also partially dephosphorylated, mainly on tyrosine residues. Only a single tyrosine residue, located in the ATP-binding site of the kinase, was phosphorylated. When this site was mutated to a phenylalanine, which cannot be phosphorylated, then p34^{cdc2} was activated prematurely and advanced the cell into mitosis. These observations indicate that during most of the cell cycle p34^{cdc2} kinase activity is inhibited by phosphorylation of the tyrosine residue, blocking utilization of ATP by the enzyme. As a consequence of *cdc25⁺* activity, the tyrosine residue becomes dephosphorylated in late G2, leading to activation of the kinase and onset of mitosis. The timing of this activation is determined at least in part by the level of the *cdc25⁺* gene product p80^{cdc25}. When overexpressed, p80^{cdc25} advances cells into mitosis, and the levels of p80^{cdc25} and *cdc25⁺* transcripts vary during the cell cycle, peaking just before mitosis. Post-translational modification may also be important for p80^{cdc25} regulation because the protein is phosphorylated.

Tamar Enoch has found that the p34^{cdc2} and p80^{cdc25} components of the mitotic control provide a link between the completion of S-phase and the onset of mitosis. Mutants either altered in *cdc2⁺* so that they do not require *cdc25⁺* for p34^{cdc2} activation, or which contain elevated levels of p80^{cdc25}, enter into mitosis even if DNA replication has been inhibited. These results indicate that in wild-type cells a signal is generated at the completion of S-phase which is communicated via p80^{cdc25} to p34^{cdc2}. This leads to dephosphorylation of phosphotyrosine and thus to the initiation of mitosis. The signal is sent constitutively in mutants containing elevated levels of p80^{cdc25} or which can activate p34^{cdc2} in the absence of p80^{cdc25}, and as a consequence these mutants cannot detect a failure to complete S-phase and initiate mitosis even when chromosome replication is not completed. This is an important control ensuring that cells do not divide without a full complement of

Fig. 2. Regulatory gene functions controlling p34^{cdc2} function at onset of mitosis.

chromosomes and underlies part of the basic cyclic nature of the cell cycle.

Two further genes with roles in the mitotic control have been studied by Jacqueline Hayles. The *suc1*⁺ gene product p13^{suc1} interacts with p34^{cdc2} and is inhibitory for mitotic initiation. Its precise *in vivo* function is not known but a likely role is an involvement in inactivating the p34^{cdc2} kinase at the end of mitosis. The second gene, worked on in addition by Iain Hagan, is *cdc13*⁺, which encodes a B-cyclin p56^{cdc13}. This protein is required for p34^{cdc2} kinase activation but also functions later in mitosis. Partially defective *cdc13*^{ts} mutants arrest in mitosis with high p34^{cdc2} kinase activity and condensed chromosomes but also with a microtubular cytoskeleton typical of interphase. It appears that p56^{cdc13} has some other role in addition to p34^{cdc2} kinase activation, that is necessary for complete exit from G2 and entry into mitosis. One possibility is that it is required to maintain kinase activity during the course of mitosis.

These studies are beginning to give an outline account of the molecular mechanisms involved in fission yeast

cell cycle controls, particularly at the onset of mitosis. Similar mechanisms are also operative in multicellular eukaryotes. This was first established by Melanie Lee, who isolated the human *CDC2* homologue by complementation, using a human cDNA expression library transformed into a *cdc2*^{ts} mutant. The success of this complementation approach demonstrates that important elements of cell cycle control are highly conserved in all eukaryotes. Replacement of the yeast *cdc2*⁺ gene by its human homologue has been carried out by Stuart MacNeill and has generated a strain we have called the 'humanized yeast'. This strain has allowed genetical studies to be carried out on the human gene in yeast which show that it responds to the *wee1*⁺ and *cdc25*⁺ genes in a similar way as does the yeast *cdc2*⁺ gene. This is suggestive that analogues of other fission yeast mitotic control genes will also be found in human cells. This complementation approach is likely to be of general use for the study of many problems of cell and molecular biology in multicellular eukaryotes. It is perhaps extraordinary that yeasts and humans should control their division in essentially the same way, despite having diverged around 1000 million years ago. It illustrates the common links to be found in all living organisms whether they be microbes or mammals. This was nicely emphasized in a lecture given by Guido Pontecorvo at a recent symposium, *Genetics and Society*, in Leicester. He argued, given that 'the whole biosphere shares a gigantic gene pool, we should become more respectful of all living things'. I couldn't agree more.

To end on a light note. I am often asked what pombe beer tastes like. The answer can be found in a travel book, *Jaguars Ripped My Flesh*, by Tim Cahill; the book has a chapter called 'Pombe Wisdom' in which the taste is described in lurid detail. I quote: 'Pombe is a product of the tiny African country of Rwanda. I'm not sure how pombe is made, but what I do know is consistent with its revolting taste: it involves mashing up rotten bananas with bare feet and burying the resultant mess in a cask for an undetermined amount of time. After the pombe is exhumed, it is poured into litre bottles . . . an unappetising black sludge forms at the bottom of the bottle and along the sides. The stuff tastes like death . . .' and so on. The conclusion is clear, work on pombe but do not drink it!