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The Wellcome Lecture, 1992: Cell Cycle Control

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Phil. Trans. R. Soc. Lond. B 1993 **341**, 449-454
doi: 10.1098/rstb.1993.0127

The Wellcome Lecture, 1992. Cell cycle control

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CONTENTS

SUMMARY

Genetic analysis using the fission yeast has provided a powerful methodology to investigate the eukaryotic cell cycle and its control. The onset of M-phase in fission yeast is controlled by a regulatory gene network which activates the $p34^{cdc2}$ protein kinase encoded by the *cdc*2^{+} gene. The coupling of M-phase to the completion of S-phase also works through $p34^{cdc2}$. A similar network is operative in vertebrate cells. Future work will focus on the controls regulating onset of S-phase and on the mechanisms by which a cell duplicates itself in space during division.

1. INTRODUCTION

Understanding the process of cell reproduction is an important biological problem. The reproduction of cells underlies the growth of all living organisms, including the population increase seen in unicellular organisms and the development of multicellular organisms. The ability to reproduce is a universal characteristic of life which is exhibited at its simplest level with the division of the cell. Cell reproduction is also a microcosm of development because, as in all developmental processes, it requires an orchestrated series of events, organized both in time and in space within the cell. When the controls over cell reproduction are disturbed in multicellular organisms, this can lead to a variety of pathological conditions, most notably cancer. In this lecture I want to consider the basic controls which regulate the reproduction of eukaryotic cells. This discussion will focus largely on work dealing with the simple unicellular eukaryote, fission yeast, and how this work can be extended to vertebrate organisms.

2. ESSENTIAL FEATURES OF CELL REPRODUCTION

The cell reproduces itself during the cell cycle. In order for the two cells generated at each cell division to survive, the processes of the cell cycle must ensure that the two daughters receive an adequate share of all necessary components. Of particular importance is the hereditary material encoded by the chromosomes. These are present in low copy number within the cell, and thus special mechanisms are required to bring about accurate chromosome duplication and segregation into the two daughter cells.

Chromosomal duplication occurs during S-phase, the phase of the cell cycle when the DNA molecules become replicated. The precise copying of the DNA molecules during replication is a direct consequence of the double helix structure composed of two complementary DNA strands. Much progress has been made in understanding the enzymic machinery responsible for DNA replication, but the controls regulating the onset of S-phase remain more obscure. Segregation of the duplicated chromosomes occurs during the second major phase of the cell cycle, M-phase, or mitosis in the mitotic cycle and meiosis in the meiotic cycle. Precise chromosomal segregation requires a complex series of cellular morphogenetic activities. Firstly, the cell establishes bipolarity so that the duplicated chromosomes can migrate to the different compartments within the cell destined to become the distinct regions of the daughter cells. Bipolarity is established

Phil. Trans. R. Soc. Lond. B (1993) **341,** 449–454
Printed in Great Britain

by reorganizing the cellular cytoskeleton, and involves the generation of bipolar microtubular organizing centres which will eventually generate the M-phase spindle. Secondly, the duplicated chromosomes become orientated with respect to each other and the bipolar organized cell, such that the two members of each duplicated pair can segregate away from each other to the two daughter cell compartments. Thirdly, the chromosomes move away from each other to the two daughter compartments. These activities are complex, and a full description is not yet available, although many of the molecules and their interactions have been identified. In contrast, the controls regulating the onset of M-phase are now quite well understood and their description will form a major part of this lecture.

The processes of S and M phase are common to all normal cell cycles because it is essential that each daughter cell receives a full chromosomal complement. There are also many other components which must be shared between the daughter cells. However, the majority of these are present in high copy and so no special mechanisms are required for their segregation. It is sufficient that their levels increase roughly in proportion to the general increase in mass of the cell, and that they are distributed evenly throughout the cell. The process of cell division will then bring about approximately equal partitioning between the daughter cells. Other components present in moderate to low copy number such as the organelles, may require special mechanisms but these have not been well studied. One possibility includes fragmentation to bring about a transient high copy number and dispersal throughout the cell, as occurs with the membrane components of the golgi which undergo breakdown during M-phase (Warren 1985). Another possibility is an association with the chromosomal segregation apparatus as may occur with the mitochondria in certain cell types (Wilson 1925).

3. GENETICS AS A METHODOLOGICAL PROCEDURE

Genetics provides a very powerful procedure for investigating complex problems such as the cell cycle. The procedure is to isolate mutants which are altered or defective in the process of interest. The mutants identify genes involved in the process, and these genes can then be cloned either by mapping and chromosomal walking, or by complementation of the mutant defect. This second approach can only be used if the organism being studied is transformable at high efficiency allowing gene banks to be introduced effectively. Having the cloned gene available enables a molecular investigation of the process to take place. The gene can be sequenced, possibly identifying aspects of it biochemical function, and antibodies can be raised against the gene product furthering the biochemical analysis. With this procedure the investigator can move from no or little understanding of the process towards eventually a complete molecular description. However, crucial to the procedure is the identification of the initial mutants which should be specifically and directly altered or defective in the process of interest.

This procedure has been applied with considerable success to the cell cycle. Initial work with the bacterium *Escherichia coli* was followed by a major study initiated by Hartwell using the budding yeast (see review in Hartwell 1974). He and his collaborators identified *c*ell *d*ivision *c*ycle (*cdc*) genes which are required to complete the cell cycle. Subsequent work was carried out using the fission yeast *Schizosaccharomyces pombe* (Nurse *et al.* 1976; Nasmyth & Nurse 1981), a simple unicellular organism with only four times the DNA content of *E. coli*. Despite this simplicity the fission yeast is a typical eukaryote. In particular, as shown by Mitchison (1971), its cell cycle is organized in a normal eukaryotic fashion, with distinct S and M phases separated by G1 and G2 gaps. Fission yeast is ideal for both classical and molecular genetical analysis, and the ability to transform at high efficiency allows genes to be cloned by complementation of the appropriate mutant defects.

Collections of cdc mutants defective in cell cycle progression have been made using fission yeast by screening for mutants unable to undergo cell division when incubated at a restrictive temperature (Nurse *et al.* 1976; Nasmyth & Nurse 1981). These mutants are specifically blocked in cell cycle progression rather than being defective in some more general growth process. They continue to grow at the restrictive temperature generating highly enlarged cells, which are unable to divide. Many cell cycle genes have been identified using such mutants. These genes are required for all stages of the cell cycle, including progression through the S and M phases as well as through the G1 and G2 gaps.

4. THE MITOTIC CONTROL

In order to characterize the controls regulating the reproduction of the fission yeast cell it is necessary to identify which of the cell cycle genes are concerned with controlling cell cycle progress. Such genes are expected to be important for regulating the overall rate of progression of the cell through the cell cycle to division. Mutants altered in the regulatory genes could advance cells into division prematurely by speeding up the rate of cell cycle progression. Because they do not affect the cellular growth rate, this advancement results in cells dividing at a small size. Mutants of this phenotype have been isolated and are called wee to describe their small size and Scottish origin! (Nurse 1975; Thuriaux *et al.* 1978; Nurse & Thuriaux 1980)

Analysis of the wee mutants indicates that they are advanced into mitosis prematurely and as a consequence have a shortened G2. Initial work identified four major genes *cdc*2$^+$, *cdc*25$^+$, *wee*1$^+$, and *cdr*1$^+$/*nim*1$^+$, which act together in a network regulating the initiation of mitosis (Nurse & Thuriaux 1980; Russell & Nurse 1986; Russell & Nurse 1987*a*,*b*). The *cdc*2$^+$ gene is the key element in this control. It encodes a 34 kDa protein kinase p34^{cdc2}, the activity of which rises to a peak in late G2 to bring about mitotic onset

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(Simanis & Nurse 1986; Gould & Nurse 1989; Moreno *et al.* 1989). The rise in activity is regulated by the balance between the *wee*1^+ encoded protein kinase p107^{wee1}, and the *cdc*25^+ encoded protein phosphatase p80^{cdc25}. These two gene products determine the phosphorylation state of the tyrosine residue Y15 in the p34^{cdc2} kinase (Gould & Nurse 1989; Gould *et al.* 1990; Moreno & Nurse 1991). The Y15 residue is located in the ATP and protein substrate binding region of the p34^{cdc2} kinase. When Y15 is phosphorylated the kinase is inactive and when it is dephosphorylated the kinase becomes activated. The balance of activities of p107^{wee1} and p80^{cdc25} results in dephosphorylation of the phosphorylated Y15 residue in late G2 leading to activation of the p34^{cdc2} kinase.

In order for p34^{cdc2} to become active it must be complexed with the B cyclin p56^{cdc13} encoded by *cdc*13^+ (Hagan *et al.* 1988). The level of p56^{cdc13} oscillates periodically once each cell cycle with a peak level at mitosis (Moreno *et al.* 1989). Sufficient p56^{cdc13} for p34^{cdc2} kinase activation is present early in the cell cycle and so the p56^{cdc13} level does not normally influence the rate of progression into mitosis. However, the degradation of p56^{cdc13} at the end of mitosis appears to be responsible for the drop in p34^{cdc2} kinase activity which occurs at this time (Murray *et al.* 1989); the fall in kinase activity is necessary for mitotic exit and entry into G1. Other genes which influence the mitotic control have also been identified. *mik*1^+ appears to contribute a minor protein kinase activity similar to p107^{wee1}, whilst *pyp*3^+ encodes a minor activity phosphatase activity similar to p80^{cdc25}. *nim*1^+/*cdr*1^+, *pyp*1^+ and *pyp*2^+ have more peripheral roles regulating p80^{cdc25} and p107^{wee1} activities (reviewed in Labib & Nurse 1993). The overall mitotic control responds both to cell mass, and to cellular growth rate. Before the p34^{cdc2} kinase becomes activated the cell must attain a critical mass, the precise level of which is modulated by the cellular growth rate. It is likely that the several functions involved both centrally and peripherally in the mitotic regulatory network are concerned with monitoring cell mass and growth rate and coupling this information to the mitotic control.

5. COUPLING M-PHASE TO S-PHASE

M-phase normally occurs only after S-phase is completed. This ensures that the chromosomes are not segregated before their proper duplication. If this were to occur then it would be lethal for the cell. When DNA replication is blocked using a chemical inhibitor such as hydroxyurea or a cdc mutant which blocks in G1 or S-phase, then the subsequent mitosis is also blocked. This means that the cell is able to monitor whether S-phase is completed or not and to communicate this information to the components necessary for M-phase to prevent this from taking place. This signalling control is an example of a checkpoint essential to ensure orderly and successful progression through the cell cycle (Hartwell & Weinert 1989).

The checkpoint control coupling M-phase to S-phase works through the p34^{cdc2} protein kinase (Enoch & Nurse 1991; Enoch *et al.* 1991). Mutants in *cdc*2^+ which do not require dephosphorylation of the phosphorylated Y15 residue such as F15, or mutants which reduce the phosphorylation state of Y15 such as cells overproducing p80^{cdc25}, do not block mitosis when S-phase is blocked. This indicates that the restraining signal blocking mitosis works through p34^{cdc2}, probably by influencing the Y15 phosphorylation state. Presumably a signal is generated by the nucleus blocked in S-phase which is communicated to the p34^{cdc2} kinase preventing its activation and thus blocking mitotic onset. When S-phase is complete the restraining signal is lost and so the cell can activate p34^{cdc2} and enter mitosis.

New genes which play a role in the checkpoint control coupling mitosis to S-phase have been identified (Enoch *et al.* 1992). These have been found because mutants defective in these genes can enter mitosis even when S-phase is blocked with hydroxyurea. The DNA is unreplicated and thus the nucleus cannot divide although it is often cut by the septum generated during cytokinesis at the end of mitosis. This leads to a dramatic drop in cell viability and so these mutants are *h*ydroxy*u*rea *s*uper-sensitive and as a consequence have been given the name hus. The hus genes defined by these mutants are thought to be involved in various steps of the checkpoint control, including the generation of the signal by the nucleus blocked in S-phase, the transduction of the signal, and the coupling of this signal to the p34^{cdc2} protein kinase.

A number of hus mutants are also radiation sensitive, and indeed several of the hus genes are identical to previously identified rad genes (Enoch *et al.* 1992). When cells are irradiated causing DNA damage, the subsequent mitosis is blocked until the DNA damage is repaired. This block due to DNA damage is not seen in the rad/hus mutants, an observation which implies that the signalling systems activated by incompletely replicated DNA and by damaged DNA must in part overlap. This could be explained if replication complexes are present in both cells blocked in S-phase and undergoing DNA repair, and if the presence of such complexes are part of the signalling system.

6. M-PHASE CONTROL IN VERTEBRATE CELLS

The M-phase control system worked out in fission yeast is also broadly applicable to vertebrate cells (Nurse 1990). The first line of evidence used to establish this general applicability was the identification of a human homologue of the *cdc*2^+ gene. The usual rationale employed for identifying a gene from a distantly related organism homologous to a known gene, utilizes the structural similarity between the two genes, either at the level of DNA sequence or protein structure. Searches for similarities in DNA sequence can be made using reduced stringency hybridization, or the polymerase chain reaction if it is known which sequence motifs are likely to be conserved. Genes encoding proteins with similar structures can be found using an *E. coli* expression library together with antibodies raised against the protein of interest. The

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antibodies detect clones expressing proteins containing epitopes related to those found in the protein encoded by the known gene and thus sharing some similarity in structure. The difficulty with these approaches is that they detect genes which are structurally similar but not necessarily homologous, in the sense that they may not be functionally equivalent. This is a particular problem when the gene of interest is a member of a family of structurally related but functionally unrelated genes. This is the case for the *cdc*2$^+$ gene which encodes a protein kinase, many examples of which exist in vertebrate cells.

To overcome this difficulty the human *CDC2* gene was cloned on the basis of functional equivalence by using complementation of a fission yeast cdc2^{ts} mutant (Lee & Nurse 1987). A human cDNA library that could be expressed in fission yeast cells was transformed into a cdc2^{ts} mutant and clones selected which grew at the restrictive temperature. This approach yielded the human *CDC2* gene which can fully substitute for the fission yeast gene even though the level of the human p34^{CDC2} protein in the cell is no more than that of the fission yeast p34^{cdc2} protein. The transformed cells have identical generation times to wild-type cells and there are only small differences in the properties of the mitotic control system. Comparison of the human and fission yeast gene sequences established that they both encoded protein kinases of 34kD molecular weight, differing in length by only one amino acid, and shared an overall identity of 63% in amino acid sequence. Subsequent work has indicated that there are a number of other *cdc*2$^+$ related genes in mammalian organisms, which are similar structurally but are not functionally equivalent as they fail in the cdc2^{ts} complementation assay. These other proteins encoded by cdk genes vary in similarity from 40–60% in amino acid sequence to the original *cdc*2$^+$ gene.

The fact that the human and yeast genes are functionally equivalent strongly argues that the basic organization of cell cycle control is similar in human cells to that in yeast cells, and thus is likely to be conserved in all eukaryotes (Nurse 1990). Further support for this view has come from a second line of approach. Masui described maturation promotion factor or MPF which induced maturation of Xenopus oocytes into mature eggs (Masui & Markert 1971). The maturation process involves promotion of the oocyte blocked in the G2 phase of the cell cycle to enter M-phase, the cell cycle phase in which egg cells are arrested. Therefore MPF promotes entry into M-phase in a fashion analogous to the premature entry into mitosis promoted in the wee mutants. Initial assays of MPF activity were established by injecting the contents of M-phase cells into G2 blocked oocytes and monitoring egg maturation. Assays were then developed using oocyte extracts which enabled MPF to be biochemically purified (Lohka *et al.* 1988). MPF contained two proteins, and using a combination of antibody and micro-sequencing work using both *Xenopus* and starfish oocytes one was shown to be p34^{cdc2} (Gautier *et al.* 1988) and the other cyclin B (Labbe *et al.* 1989*a*,*b*; Gautier *et al.* 1990). These studies established that a complex of p34^{cdc2} with B-cyclin is responsible for bringing about the onset of M-phase in multicellular Metazoa including vertebrates.

Genetic work in fission yeast and biochemical work in oocytes and eggs have converged to identify the same molecular components as being important for bringing about M-phase. Further work has also identified homologues of *wee*1$^+$ and *cdc*25$^+$ in vertebrate cells and has shown that Y15 phosphorylation plays a key role in M-phase regulation in these higher eukaryotes. However, it is likely that the cell cycle controls are more elaborate in multicellular organisms given the requirement for a more sophisticated regulation of cell reproduction during development and morphogenesis. Consistent with this notion, phosphorylation of T14 which, like Y15 is located in the ATP and protein substrate binding region of the p34^{CDC2} protein kinase, is also important in M-phase regulation in vertebrate cells (Norbury *et al.* 1991).

7. ONSET OF M-PHASE

The above studies establish that the basic mechanism bringing about M-phase in eukaryotic cells requires the p34^{cdc2}/Cyclin B protein kinase complex. To understand how the major processes of M-phase are brought about it is important to identify the substrates which become phosphorylated at M-phase by this protein kinase. Several potential substrates have been proposed, the phosphorylation of which may be of relevance for the major processes of M-phase (see reviews Moreno & Nurse 1990; Norbury & Nurse 1992). A class of proteins identified as substrates are the lamins, which are components of the nuclear envelope. Phosphorylation of lamins by p34^{cdc2} contributes to the breakdown of the nuclear envelope which occurs during M-phase in higher eukaryotic cells. Another candidate is H1-histone, which becomes hyperphosphorylated at M-phase and has long been proposed as important for chromosome condensation at M-phase. In fact very early studies using Physarum implicated a H1 histone kinase in the control of mitosis which turned out to be p34^{cdc2} (Langan *et al.* 1989). Immunofluorescent and fractionation studies of cells have shown that p34^{cdc2} is associated with cytoskeleton elements such as the microtubular organizing centres (Bailly *et al.* 1989). This may be because p34^{cdc2} phosphorylates components located at these sites which are necessary for the reorganization of the cytoskeleton which occurs at M-phase. Further biochemical studies should be illuminating about the direct or indirect role of p34^{cdc2} in the mechanisms establishing bipolarity, generating chromosome orientation, and bringing about chromosome movement which are central to the process of M-phase.

8. FUTURE WORK

In contrast to the controls regulating the onset of M-phase those operative over S-phase remain more obscure. An important aspect of S-phase control in yeast is at Start, the point in G1 when the cell

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becomes committed to the mitotic cell cycle. First described by Hartwell in budding yeast (Hartwell 1974) a similar Start control also operates in fission yeast (Nurse & Bissett 1981). Before Start, cells are developmentally uncommitted in the sense that they can be diverted from the mitotic cycle to undergo alternative developmental programmes such as conjugation. After Start, events leading to S-phase are initiated, including transcription of the genes encoding the histones and ribonucleotide reductase. In fission yeast two gene products are required to complete Start, those encoded by *cdc*2$^+$ and *cdc*10$^+$ (Nurse & Bissett 1981). The *cdc*2$^+$ gene is unique in being required twice during the cell cycle at Start and at the onset of M-phase. Its role at Start is not understood, particularly as only very low protein kinase activity has been detected at this time of the cell cycle (Moreno *et al.* 1989).

More progress has been made with working out the role of p87^{cdc10} which is encoded by the *cdc*10$^+$ gene. p87^{cdc10} (Aves *et al.* 1985; Simanis & Nurse 1989) is proposed to be part of a transcriptional complex binding to a DNA sequence motif called the mlu1 box (MCB) (Lowndes *et al.* 1992). This motif is found in the promotor region of the *cdc*22$^+$ gene encoding ribonucleotide reductase which is regulated periodically during the cell cycle. The MCB element endows cell cycle periodicity on the transcription of reporter genes, with transcriptional activation occurring as cells proceed through Start. It is likely that passage through Start leads to activation of this transcriptional complex resulting in expression of key genes required for the onset of S-phase.

Future work will focus on two aspects of Start regulation. Firstly what are the important gene targets of the transcriptional complex and how do these bring about the initiation of DNA replication? Ribonucleotide reductase is one target but is not likely to be important in the initiation process. A more promising candidate is the *cdc*18$^+$ gene product which is a major downstream target of the p87^{cdc10} containing complex. Futher work should identify other major targets, and subsequent biochemical investigation should be illuminating about the molecular mechanisms underlying the initiation of DNA replication. The second aspect of Start regulation of particular interest is what controls the onset of Start. Before cells can pass through Start they must attain a minimal cell mass and also complete the previous mitosis. Therefore the regulatory controls must monitor these two requirements at Start. One gene involved in this monitoring is *rum*1$^+$. Ectopic expression of *rum*1$^+$ results in *r*eplication being *u*ncoupled from *m*itosis accounting for the name of the gene. Deletion of the gene eliminates the pre-Start G1 internal and reduces the cell mass requirement at Start.

To date work on the cell cycle has focused on temporal controls, that is how the events of the cell cycle are organized in time. Another important problem is the process by which the cell duplicates itself in space. When a cell undergoes mitosis and cell division its whole organization has to be duplicated. One aspect of this is the duplication of the microtubular organizing centre (MTOC) which occurs at mitosis establishing bipolarity within the cell. At division each daughter cell inherits an MTOC which is partly responsible for the spatial re-organization. To start work on this problem my laboratory has isolated mutants in fission yeast with an altered cell shape. Hopefully by understanding the controls which regulate overall cell shape and organization, it may be possible to address how the cell duplicates itself in S-phase during the process of cell reproduction.

I thank the many people who have worked in my laboratory over the years and who have carried out much of the work dealt with in this lecture. My laboratory has received most of its support from ICRF and MRC.

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Lecture delivered 21 January 1993

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