Perspectives

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Twenty-Five Years of Cell Cycle Genetics

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IN the last few years, a unified view of the eukaryotic cell cycle has arisen that had its origins in several diverse areas of research including the activation of amphibian oocytes, protein synthesis in cleavage-stage embryos, histone phosphorylation, and yeast genetics. My own involvement began with a genetic analysis of essential functions in the yeast *Saccharomyces cerevisiae* that soon focused on the control of the cell cycle. The historical development of my concepts about the cell cycle, related here, is likely to result in a different perspective from that of those who began their work with maturation promoting factor, cyclins, H1-kinase or even the yeast *Schizosaccharomyces pombe*; hopefully, we would all arrive at a similar current picture. Other recent reviews emphasize the developments of the last few years in greater detail (DUNPHY and NEWPORT 1988; HARTWELL and WEINERT 1989; MINSHULL *et al.* 1989; MURRAY and KIRSCHNER 1989a; LEWIN 1990; NURSE 1990; ENOCH and NURSE 1991; MALLER 1991).

Detecting cell cycle mutants: Although a few cell division cycle (*cdc*) mutants of *S. cerevisiae* were first recognized among a larger collection of temperature-sensitive lethal mutants in 1967 (HARTWELL 1967), the application of time-lapse photomicroscopy in 1970 (HARTWELL, CULOTTI and REID 1970) resulted in the rapid identification of many such mutants. Temperature-sensitive *cdc* mutants were defined as mutants that arrested division at a unique stage of the cell cycle regardless of their stage at the time they were shifted from permissive to restrictive temperature. The detection of *cdc* mutants was aided in *S. cerevisiae* by the observation that all of the cells with the same *cdc* mutation arrested division with the same parent-bud morphology at the restrictive temperature.

About 10% of all temperature-sensitive mutants of *S. cerevisiae* were *cdc* mutants, suggesting that there may be as many as 500 genes with stage-specific functions in the eukaryotic cell (HARTWELL *et al.* 1973). However, the number of *CDC* genes that could be found easily by analyzing temperature-sensitive mutants plateaued at around 70, a result that probably reflects the difficulty of obtaining temperature-sensitive alleles of many gene products and the fact that many genes are present in redundant copies. Through the advent of new approaches, many new *CDC* genes are currently being identified in a variety of organisms, especially *S. cerevisiae*, *S. pombe*, *Aspergillus nidulans* and *Drosophila melanogaster*, and it is likely that several hundred will be known within the next few years.

Dependent order of events: The phenotypes of the *cdc* mutants revealed a fundamental fact about the control of the cell cycle, namely that the execution of late events in the cell cycle depended on the prior completion of early events (HARTWELL *et al.* 1974; NURSE, THURIAUX and NASMYTH 1976), a condition that defines a *dependent pathway* or *dependent events*. Although more than one pathway of events was evident, most of the mutant phenotypes could be explained by a relatively small number of pathways. For example, most of the mutants with defects in spindle morphogenesis, DNA replication, chromosome segregation or nuclear division are organized into a single dependent pathway. The functions executed by the heat-sensitive *cdc* gene products were ordered with respect to the functions inhibited by stage-specific inhibitors (HEREFORD and HARTWELL 1974; HARTWELL 1976; WOOD and HARTWELL 1982) or with respect to cold-sensitive *cdc* gene products (MOIR and BOTSTEIN 1982); these studies revealed that the dependent order of cell cycle events was a result of an underlying order of gene product function. The view of the yeast cell cycle generated by these observations was that of a cascade of events whose order was invariant because late functions could not occur until preceding early functions had been completed. Less comprehensive studies with stage-specific inhibitors

and with mutants of metazoan somatic cells were consistent with this view.

Contrasting embryonic cell divisions: The cell divisions of the early Xenopus embryo presented a striking contrast to this view of a cascade of dependent events. Mitosis does not depend on DNA replication as it does in many other cells because inhibition of DNA synthesis does not prevent nuclear division (KIMELMAN, KIRSCHNER and SCHERSON 1987; RAFF and GLOVER 1988). Moreover, inhibition of mitosis does not prevent successive rounds of DNA replication (KIMELMAN, KIRSCHNER and SCHERSON 1987) as it does in many other cells. The early embryos of Drosophila (RAFF and GLOVER 1988) and sea urchins (NISHIOKA, BALCZON and SCHATTEN 1984) display a similar uncoupling of cell cycle events. Furthermore, the activated but enucleated Xenopus egg exhibits contractions with the same periodicity as the divisions of a nucleated egg, suggesting the presence of a cytoplasmic clock that controls cell divisions (HARA, TYDEMAN and KIRSCHNER 1980).

The unified view: How were these differences to be reconciled? On the one hand the cell cycle of yeast and most other eukaryotic cells appeared to be a cascade of events, each succeeding event depending on the former (the domino model), while the cell cycle of the Xenopus embryo seemed to be a number of independent events possibly controlled by a central clock (the clock model) (MURRAY and KIRSCHNER 1989a).

Despite these striking apparent differences in how cell cycles could be controlled, certain observations hinted that the two cell cycles might share elements of both models. For example, in Xenopus (NEWPORT and DASSO 1989) the control of the cell cycle changes during development: at later stages, mitosis comes to depend on the completion of DNA replication. Thus, the two types of cell cycle organization can exist within a single organism, a fact which suggests that the two modes may not be fundamentally different. Furthermore, one *cdc* mutant of yeast displays periodic behavior, suggesting the presence of a cell cycle clock (HARTWELL 1971); *cdc4* mutants arrest the nuclear cycle at the restrictive temperature but continue multiple rounds of budding with a periodicity similar to the interval between normal cell cycles. Hence, yeast seems to have a clock as well as a cascade of events.

Recent work has suggested a synthesis of these two models. The cell cycle of all cells is now thought to be driven by a protein kinase that exhibits cyclic behavior and is the biochemical basis of the "clock" evident in both Xenopus and yeast. In some embryonic cell cycles this clock activates successive events in turn and the events do not depend on one another. In the cell cycles of most other eukaryotic cells, the same kinase activates events in the cell cycle but, in addition, control circuits are present which prevent late events from occurring until early events have been completed. I will consider the evidence for the kinase clock first and then the evidence for the control circuits that enforce dependent pathways.

The clock: The genetic analysis of the clock began with the identification of mutants in genes that occupied a central role in cell cycle control. One gene, *CDC28*, identified the first function (termed "Start") in the sequence of dependent events in the *S. cerevisiae* mitotic cell cycle (HEREFORD and HARTWELL 1974). *CDC28* was necessary to activate two independent pathways, one leading to bud emergence and cytokinesis and the other to DNA replication and nuclear division (HARTWELL *et al.* 1974). In addition to occupying the first step in the cycle, it was also the focus for cell cycle control both by pheromones (HEREFORD and HARTWELL 1974) and by nutrients (JOHNSTON, PRINGLE and HARTWELL 1977).

A central control gene in *S. pombe* was identified as the *CDC2* gene. Attention was focused on this gene in *S. pombe* because it was essential for mitosis and because certain alleles altered cell size at mitosis (NURSE and THURIAUX 1980). Like the *CDC28* gene of *S. cerevisiae*, it appeared to be involved in the integration of growth and division. However, dramatic differences between the apparent functions of *CDC28* of *S. cerevisiae* and *CDC2* of *S. pombe* initially obscured their relationship. *CDC28* was essential in G1 while *CDC2* was essential in G2. However, once again certain facts hinted that these apparent differences might not be fundamental. The *CDC2* gene of *pombe* did affect G1 when cells were emerging from stationary phase (NURSE and BISSETT 1981) and one specific allele of the *CDC28* gene of *cerevisiae* was reported to arrest in G2 (PIGGOTT, RAI and CARTER 1982).

Three other lines of research ultimately proved to be related to these two control genes of the yeasts. Fertilized or otherwise activated eggs of Xenopus contained a cytoplasmic factor, maturation promoting factor, that could stimulate unactivated eggs to mature and begin cleavage divisions. This activity appeared periodically in cleaving eggs at about the time of mitosis (GERHARDT, WU and KIRSCHNER 1984). Another protein, cyclin, was observed to be destroyed and resynthesized in cleavage embryos of marine invertebrates with the periodicity of the cell cycle and it was guessed that this protein might be related to the maturation promoting factor (EVANS *et al.* 1983). In addition, studies of protein phosphorylation during the cell cycle of mammalian cells showed that several proteins, including histone H1, were phosphorylated at mitosis, and this led to a search for the histone H1 kinase.

In what must be one of the most unifying discoveries in cell biology, biochemical studies demonstrated that

the *CDC28* gene product of *S. cerevisiae*, the *CDC2* gene product of *S. pombe*, and the maturation promoting activity of Xenopus were all related serine-threonine protein kinases (NURSE 1990); genetic studies have shown that the kinases from *S. pombe*, *S. cerevisiae* and humans are functionally homologous (BEACH, DURKACZ and NURSE 1982; BOOHER and BEACH 1986; LEE and NURSE 1987). The active kinase is composed of the p34 gene product (*CDC2pombe/CDC28cerevisiae* protein) and a cyclin protein. I will refer to this kinase activity as the *CDC2/CDC28* kinase. Elegant experiments of MURRAY and KIRSCHNER (1989b), using cell free extracts from Xenopus oocytes that undergo cyclic DNA replication and mitosis *in vitro*, demonstrated that cyclin synthesis is necessary and sufficient to drive successive cell cycles. Hence the Xenopus oscillator is due in part to the periodic synthesis and degradation of cyclin coupled with the activation and inactivation of the *CDC2/CDC28* kinase.

A great deal of research activity focused on the *CDC2/CDC28* kinase family is revealing considerable complexity. Many members of the family have been identified (for example, four in *S. cerevisiae*) (REED, HADWIGER and LORINCZ 1985; COURCHESNE, KUNISAWA and THORNER 1989; ELION, GRISAFI and FINK 1990; LEVIN *et al.* 1990) and it is unclear at the present time how many kinases of this family are involved in controlling the cell cycle in any one organism. Furthermore, the cyclins also constitute a large family, seven being known in *S. cerevisiae* (HADWIGER *et al.* 1989; SURANA *et al.* 1991). With numerous kinases and cyclins, the possibilities for different combinations are enormous. The complexity of the system is greater still because several proteins control the activity of the kinase by phosphorylation and dephosphorylation (NURSE 1990; MALLER 1991). In addition, both the synthesis and the activity of the cyclins are controlled (CHANG and HERSKOWITZ 1990; ELION, GRISAFI and FINK 1990; CROSS and TINKELENBERG 1991; DIRICK and NASMYTH 1991). One of the central questions to be addressed is how many different steps in the cell cycle are controlled by a member of the *CDC2/CDC28* kinase family. At the present time we know that the *CDC28* product of *S. cerevisiae* and the *CDC2* product of *S. pombe* are required both in early G1, at Start, and at Mitosis; three cyclins function in *S. cerevisiae* G1 (RICHARDSON *et al.* 1989) and four in G2 (SURANA *et al.* 1991). In Xenopus, distinct kinases are required for DNA replication on the one hand and for mitosis on the other (FANG and NEWPORT 1991).

Checkpoints: Now, if we accept the idea that all cells employ the same *CDC2/CDC28* kinase to activate key steps of the cell cycle, then it appears as if all cell cycles are basically the same. However, there remains the issue of why in some cases late events depend on early events and in other cases they do not.

Insight into this paradox has come primarily from studies on the dependence of mitosis upon prior DNA replication (or upon the repair of DNA damage). In some cases this dependence has been overcome by fusing cells at different stages, by adding inhibitors, or by mutations. Fusing M phase mammalian cells with G1 cells causes the G1 nuclear membrane to break down and chromosomes of the G1 cell to condense, suggesting that replication is not necessary for mitosis but rather that the cytoplasm of the cell must reach a "mitotic" state (RAO and JOHNSON 1970). Adding caffeine causes mammalian cells to enter mitosis prior to completing DNA replication (SCHLEGEL and PARDEE 1986). The *tsBN2* mutation of mouse cells (NISHIMOTO *et al.* 1981), the *bimE7* mutation of Aspergillus (OSMANI *et al.* 1988), or eliminating the *RAD9* gene of *S. cerevisiae* (WEINERT and HARTWELL 1988) or the *wee1* and *mik1* genes of *S. pombe* (LUNDGREN *et al.* 1991) allows cells to enter mitosis without first completing DNA replication. These results demonstrate that the dependence of mitosis upon prior DNA replication is not intrinsic to the mitotic apparatus but rather is due to an extrinsic control mechanism. It is likely that the dependent relations between many events of the cell cycle are due to similar controls (HARTWELL and WEINERT 1989). We have termed these control points in the cell cycle "checkpoints" and we think of them as signal transduction pathways that generate an inhibitory signal in response to delayed upstream events and target this signal to the next downstream event.

We can now reconcile the domino and clock models of the cell cycle. All cell cycles may be run by the *CDC2/CDC28* kinase oscillator; in addition to this, somatic cells and eukaryotic microorganisms have checkpoint controls that feed forward to the next event to ensure that it does not occur if the previous event has not been completed. The early embryonic cell cycles of Xenopus and Drosophila appear to lack some checkpoint controls; these are imposed later in development. Recent work has shown that one of these control circuits is, in fact, present in the early Xenopus embryo even though it is cryptic. Injection of an inhibitor of DNA replication will inhibit mitosis only if a large amount of DNA is also injected, suggesting that the lack of dependence observed in the early embryo is explained by the fact that there is not enough DNA in the large egg cell to create a sufficiently strong signal (DASSO and NEWPORT 1990).

One of the important issues for the immediate future is to determine how many such checkpoints there are in the eukaryotic cell cycle. The phenotypes of cell cycle mutants indicate that there are many dependent steps in the cell cycle. Some of these may be simply due to the fact that the upstream event

provides an essential substrate for the downstream event. Others may be due to checkpoint controls extrinsic to the events themselves. These possibilities can be resolved by genetic analysis. If a loss-of-function mutation in one gene can relieve the dependence of certain cell cycle events, then it is clear that a checkpoint exists. Recently, mutants that relieve the dependence of budding or DNA replication on completion of the previous mitosis have been found in *S. cerevisiae* (LI and MURRAY 1991; HOYT, TOTIS and ROBERTS 1991).

Another important question is whether the control circuits are really extrinsic to the events that are being controlled. Although the *RAD9* gene is dispensable for the yeast cell cycle, other mutations that relieve the dependence of mitosis on DNA replication are lethal. Is this because it is essential to have such control or because the components that mediate the control also perform other essential functions?

Finally, it will be important to determine the signals and targets of these signal transduction pathways. There is evidence that at least some of these controls act on the *CDC2/CDC28* kinase. Certain mutations in the *CDC2* gene of *S. pombe*, or in genes that control the activity of the *CDC2* kinase, relieve the dependence of mitosis upon prior DNA replication (ENOCH and NURSE 1990; LUNDGREN *et al.* 1991). Moreover, inhibiting DNA replication in cycling Xenopus extracts (with added excess DNA) inhibits mitosis and concomitantly prevents activation of MPF kinase (DASSO and NEWPORT 1990). Whether the *CDC2/CDC28* kinase controls other steps in the cell cycle in addition to Start and Mitosis and whether all of the checkpoints target the *CDC2/CDC28* kinase are also issues to be resolved in the future.

Fidelity: In addition to understanding how any biological process works, it is also of interest to understand how its precision is achieved. The fidelity of mitotic chromosome transmission in *S. cerevisiae* is quite high; cells lose or gain a particular chromosome only about once in 10^5 divisions (ESPOSITO and BRUSCHI 1982; HARTWELL *et al.* 1982; WHITTAKER *et al.* 1988). This accuracy can be compromised by perturbations in the activity of essential components of the mitotic machinery. If essential components are rate limiting for progress or are supplied in excess of other components, then dramatic increases in the rate of chromosome loss often result (HARTWELL and SMITH 1985).

Checkpoints are likely to be another important component in mitotic fidelity. Indeed, I presume that the advantage conferred by a more accurate mitosis motivated the evolution of checkpoints. Loss of the *RAD9* checkpoint decreases mitotic fidelity 10–20-fold in an unperturbed cell (WEINERT and HARTWELL 1990) and has a much greater effect if the cell is experiencing DNA damage or defects in DNA replication. Similar effects on mitotic fidelity were found for the loss of another checkpoint control (LI and MURRAY 1991). The high fidelity of the *S. cerevisiae* cell cycle may be due to many such checkpoints that delay the cell cycle whenever intrinsic errors are made in order to permit repair of these errors.

If it is true that checkpoints exist to ensure the high fidelity of mitosis, we might wonder how the early embryos of Drosophila and Xenopus came to dispense with these controls. One would think that errors during early embryonic divisions would be especially devastating. We have suggested that some early embryos have dispensed with these controls because their developmental strategies require very rapid and synchronous mitotic divisions. Checkpoints act antagonistically to these needs because they delay division to permit repair and they do so only in the subset of cells that have experienced a perturbation to the normal process.

Is there any way for these embryos to avoid the mitotic errors that would occur in the absence of cell cycle checkpoints? One method would be to wait until the early divisions are complete, survey the nuclei, and discard those that are abnormal. This idea is not as far-fetched as it may seem because it is clear that organisms can detect the number of *X* chromosomes in a nucleus, as well as autosomal aneuploidy; furthermore, embryos can develop normally after surgical removal of many nuclei. There is evidence that Drosophila embryos discard abnormal nuclei. Nuclei that have failed to separate completely from neighboring nuclei (SULLIVAN, MINDEN and ALBERTS 1990) or nuclei with a chromosome that is lagging on the metaphase plate because it is abnormally large (W. SULLIVAN, personal communication) frequently are removed from the surface of the Drosophila embryo and segregated to the interior yolk mass. An important goal for the future will be to determine how widespread among organisms is the absence of checkpoints during early embryonic divisions and what mechanisms, if any, exist for discarding abnormal nuclei.

Finally, it is almost certain that cell cycle work will inform human disease research. Intense research is currently focused on identifying oncogenes and tumor suppressor genes and in finding out how their products impinge upon the expression or activity of the *CDC2/CDC28* kinase. Furthermore, an important component in the origin of cancer is likely to be found in changes in the fidelity of mitosis that permit rapid evolution of malignant cells; the changes that lead to this infidelity may be found in perturbations to the checkpoints of the cell cycle.

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