REGULATORY GENES CONTROLLING MITOSIS IN THE FISSION YEAST *SCHIZOSACCHAROMYCES POMBE*

PAUL NURSE*

Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JT, U. K.

AND

PIERRE THURIAUX

Institute of Microbiology, University of Bern, Bern, Switzerland

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ABSTRACT

Fifty-two *wee* mutants that undergo mitosis and cell division at a reduced size compared with wild type have been genetically analyzed. The mutants define two genes, *wee1* and *cdc2*, which control the timing of mitosis. Fifty-one of the mutants map at the *wee1* locus, which is unlinked to any known *cdc* gene. One of the *wee1* alleles has been shown to be nonsense suppressible. The 52nd *wee* mutant maps within *cdc2*. Previously, only temperature-sensitive mutants that become blocked at mitosis have been found at the *cdc2* locus. The simplest interpretation of these observations is that *wee1*+ codes for a negative element or inhibitor, and *cdc2*+ codes for a positive element or activator in the mitotic control. The gene dosage of *wee1*+ plays some role in determining the timing of mitosis, but the gene dosage of *cdc2*+ has little effect. However, some aspect of the *cdc2* gene product activity is important for determining when mitosis takes place. The possible roles of *wee1* and *cdc2* in the mitotic control are discussed, with particular reference to the part they may play in the monitoring of cell size and cell growth rate, both of which influence the timing of mitosis.

CELL cycle mutants have been isolated in a number of eukaryotic organisms, including *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Aspergillus nidulans*, *Tetrahymena pyriformis* and various lines of mammalian cells (see review by SIMCHEN 1978). Most of these mutants are temperature-sensitive lethals that become blocked at a specific stage of the cell cycle when incubated at the restrictive temperature. The mutants have been useful in identifying *cdc* (cell division cycle) genes whose functions are required for the completion of the various events that make up the cell cycle, such as DNA replication, mitosis and cell division.

Some of the functions encoded by the *cdc* genes may be important in determining the rate at which the cell progresses through the complete cell division cycle. Such functions cannot be distinguished by *cdc* mutants that become blocked in

* Present address: School of Biological Sciences, University of Sussex, Falmer, Brighton, Sussex, BN1 9QG, U.K.

progress through the cell cycle because a mutant defective in any function required for the cell cycle will be unable to complete cell division under the restrictive conditions, regardless of whether or not the affected function is normally rate-limiting. However, rate-controlling functions can be identified by mutants that complete that function more rapidly than normal because a function that is not rate limiting, and is completed more rapidly, will have little or no effect on the rate at which the cell cycle is completed. But a function that is rate limiting, and is completed more rapidly, will increase the rate at which the cell cycle is completed. Such mutants have been described in *S. pombe* (NURSE 1975; THURIAUX, NURSE and CARTER 1978). These mutants are called *wee* because they undergo cell division at a reduced cell size in comparison to wild type. Temperature-sensitive *wee* mutants are available that divide at nearly the wild-type size at low temperature, but at about half that size at high temperature. Therefore, just after a shift from low to high temperature, when the *wee* phenotype is first expressed, the *wee* mutant cells traverse the cell cycle more rapidly than wild type, and consequently can be considered to be altered in a function that is normally rate-limiting for completion of the cell cycle (NURSE 1975; THURIAUX, NURSE and CARTER 1978).

wee mutant cells in steady-state growth have a longer G1 than wild type and a shorter G2. On shifting a temperature-sensitive *wee* mutant from low to high temperature, the first cell cycle event that can be seen to be advanced is mitosis. These two properties indicate that *wee* mutants are altered in the rate-limiting control determining when mitosis takes place. It has already been established that the *wee* mutants define two genes (THURIAUX, NURSE and CARTER 1978). In the present report, we have investigated the *wee* mutants further to establish the possible role the two genes may play in the control of mitosis.

MATERIALS AND METHODS

Strains and genetical procedures: All mutant strains used in this study are derived from the haploid wild-type strain with mating type h^- (strain 972) or h^+ (strain 975). Three of the *cdc2* mutants (*2–33*, *2–56*, *2–130*) are described in NURSE, THURIAUX and NASMYTH (1976). The other *cdc2* mutants (*2–L7*, *2–M26*, *2–M35*, *2–M55*, *2–M63*, *2–M72*, *2–M76*) were isolated and characterized by NURSE, NASMYTH and BISSETT (unpublished), using the procedures described in NURSE, THURIAUX and NASMYTH (1976). Twenty-six of the *wee* mutants are described in NURSE (1975) and THURIAUX, NURSE and CARTER (1978), and the isolation of an additional 26 are described in the present paper. All mutants were backcrossed at least 3 times to wild type before they were analyzed physiologically. The backcrossings were performed to minimize problems arising from secondary mutations influencing the behavior of the *wee* or *cdc* mutants.

We have used the standard genetical procedures for *S. pombe*, as described by GUTZ *et al.* (1974). The diploids were constructed using h^- *cdc*$^-$ *ade6–704* and *mei1–102 ura5–294* strains, as described in NURSE, THURIAUX and NASMYTH (1976).

Media and growth conditions. The media used in the genetical procedures have been described by GUTZ *et al.* (1974). These include malt extract agar, yeast extract glucose agar supplemented with adenine and with Phloxin B (from Sigma) and minimal agar. Phloxin B stains dead and sick cells. For all physiological experiments, strains were grown in liquid culture using the minimal medium EMM2 (MITCHISON 1970), as modified by NURSE (1975). In some experiments involving adenine auxotrophs, adenine was supplemented at 75 mg/l. Cultures were grown in a water bath at 25° or 35° with stirring.

Physiological analysis: Cell length at septation was measured using a Zeiss microscope with an eyepiece micrometer. Protein content per cell in an exponentially growing population was determined by the Folin reaction without prior acid hydrolysis (NURSE and THURIAUX 1977). Standard errors of the estimates of length and protein content were no greater than 3% of the mean.

Cell length at septation was used as a measure of the degree to which a cell was advanced or delayed in undergoing mitosis. The rationale for this is as follows: In *S. pombe*, including *wee*⁻ and *cdc*⁻ mutants, growth during the cell cycle is mostly by elongation with little change in cell diameter. There is also little change in cell density during the cell cycle (MITCHISON 1957); consequently, length is a good measure of the size of the cell. Septation takes place rapidly after mitosis is completed (MITCHISON 1970); thus cell length at septation is proportional to cell size at mitosis. Mitosis normally takes place when cells have grown to a certain size. If mitosis is initiated early, septation will take place in cells of a shorter length than normal, and, if mitosis is delayed, cells will be longer than normal at septation. Thus, cell length at septation can be used as a measure of the degree to which a cell is advanced or delayed in undergoing mitosis.

Mapping the cdc2 *locus*: A fine-structure genetic map of the *cdc2* locus was constructed by doing pairwise crosses among the various *cdc2* alleles. The distances among the alleles are expressed as the frequency of wild-type recombinants produced in the crosses, as measured by random-spore analysis. In crosses involving *cdc2* alleles that were unable to form colonies at 35°, wild-type recombinants could be easily detected by plating at 35°. In crosses involving *cdc2-1w*, which could form colonies at 35°, wild-type recombinants were detected by plating the spores at 35° on yeast extract glucose agar supplemented with Phloxin B. Under these growth conditions, wild-type colonies were pink, whereas *cdc2-1w* colonies were red. This procedure was more tedious than that used with *cdc2* alleles, and fewer spores could be analyzed. As a consequence, the map distances involving *cdc2-1w* are less precise than those involving the other *cdc2* alleles.

RESULTS

Properties of wee *mutants: evidence for a gene acting as a negative element:* Twenty-six additional *wee* mutants were isolated using the procedure described in THURIAUX, NURSE and CARTER (1978). They were all crossed to a tester strain *wee1-50*, but none of them yielded recombinants of wild-type size in qualitative random-spore analysis using the "criss-cross" method of LEUPOLD (GUTZ *et al.* 1974). It was concluded that the 26 mutants mapped at the *wee1* locus, giving a grand total of 51 when included with 25 isolated previously (NURSE 1975; THURIAUX, NURSE and CARTER 1978). At 35°, all of these *wee1* mutants had the same phenotype, undergoing septation at a cell length about half that of wild type. Their generation times were not much increased compared to wild type, but could be up to 20% longer. Four of the mutants, bearing *wee1-1*, *wee1-3*, *wee1-6* or *wee1-112*, were backcrossed three times to wild type, and the derivative strains were tested for complementation with the originally isolated *wee1-50*. The cell lengths at septation of the four *wee1*⁺/*wee1*⁻ diploids were all about 80% of that found for the wild-type diploid (lines 1 through 5 of Table 1). These lengths should be compared to those obtained with the homozygous *wee1-50* diploid and the three *wee1*⁻/*wee1-50* heteroallelic diploids (line 7 through 11 of Table 1), for these all divided at a length about 55% of that of the wild-type diploid. This result means that none of the three *wee1*⁻ alleles appeared to complement the original *wee1-50* allele, suggesting that all the alleles are mutations in a single cistron. However, this conclusion should be treated with some caution,

TABLE 1

Complementation and dominance relations of wee1 *alleles*

Strain	Cell length at septation µm	Cell length at septation %*	Mean protein content per cell pg/cell	Mean protein content per cell %*
wee1+/wee1+ diploid	23.9	100	22.6	100
wee1+/wee1-1 diploid	18.3	77	—	—
wee1+/wee1-3 diploid	20.9	87	—	—
wee1+/wee1-6 diploid	20.2	85	—	—
wee1+/wee1-112 diploid	19.5	82	19.6	87
wee1+/wee1-50 diploid	19.3	81	18.5	82
wee1-50/wee1-50 diploid	12.4	52	13.0	58
wee1-50/wee1-1 diploid	13.0	54	—	—
wee1-50/wee1-3 diploid	13.9	58	—	—
wee1-50/wee1-6 diploid	13.8	58	—	—
wee1-50/wee1-112 diploid	13.4	56	—	—

* % of wild-type diploid *wee1+/wee1+*.
Strains grown at 35° in minimal medium. The standard errors of the estimates of cell length at septation were no greater than 3% of the mean.

since the decreases in size of the heteroallelic diploids are not far from the sums of the decreases in size of the corresponding single heterozygotes.

The advancement of cells into mitosis at a small size could be due to mutations in a gene acting as either a negative or a positive element in the mitotic control. By negative element we mean one that inhibits mitosis and by positive element, one that activates mitosis. In the case of *wee1*, two lines of genetic analysis support the hypothesis that it functions as a negative element:

(1) Thirty *wee1* alleles were tested for nonsense suppressibility by crossing the original mutants with *sup3-5*, an efficient suppressor of opal nonsense alleles (KOHLI *et al.* 1979). One allele, *wee1-112*, proved to be suppressible. The original *wee1-112* mutant strain divided at a length 51% of that of *wee1+*, but in the presence of *sup3-5* the length was increased to 89% (Table 2). Since nonsense mutations usually produce completely inactive proteins, this result suggests that the *wee* phenotype is caused by a loss of activity of the *wee1* gene product. The corollary of this is that the *wee1+* gene product in a wild-type cell

TABLE 2

Suppression of wee1-112 *by an opal nonsense suppressor*

Strain	Cell length at septation µm	Cell length at septation %*
wee1+ sup3-5 ade6-704	14.7	100
wee1-112 sup3+ ade6-704	7.5	51
wee1-112 sup3-5 ade6-704	13.1	89
wee1+ sup+ ade6-704	15.0	102

* % of strain with wild-type *wee* gene and opal nonsense suppressor *wee1+ sup3-5 ade6-704*.
Strains grown at 35° in minimal medium supplemented with adenine.

acts negatively in the control circuit and delays or inhibits mitosis. The nonsense suppressibility of *wee1–112* also establishes that the gene product is a protein.

(2) If the *wee1* gene product acts positively to activate mitosis, two types of mutants could be expected: mutants with an activator that either has increased activity or is over-produced, resulting in a *wee* phenotype, and mutants with a defective activator that would be unable to undergo mitosis at all. The latter class of mutants would be common since they involve a loss of gene product activity. They would have a *cdc* phenotype since they would be unable to complete mitosis and cell division. To test for this possibility, representatives of all the known 26 *cdc* genes were crossed with *wee1–50*. All the *cdc* representatives recombined freely with *wee1–50*, demonstrating that *wee1* is not linked to any known *cdc* gene. In addition, the high frequency of *wee* mutants that map at the *wee1* gene (51 of 52 independently isolated mutants), implies that mutants result from lesions at a large number of different sites within the *wee1* gene. This was confirmed by the observation that occasional wild-type recombinants were observed in crosses between *wee1–1*, *wee1–6*, *wee1–50* and *wee1–112*. Wild-type recombinants were detected both by their failure to stain with Phloxin B and by their cell size, and occurred at a frequency of between 10 and 100 in 10^6 spores, a level that is compatible with intragenic recombination. Since the four *wee1*$^-$ mutations mapped at different sites within the *wee1* gene, it is likely that there are many *wee1* mutational sites that can result in the *wee* phenotype. This result argues that *wee* mutations result in a loss of activity of the *wee1* gene product, and thus that *wee1* acts as a negative element in the control.

Properties of cdc2 *mutants: evidence for a gene acting as a positive element:* Only a single *wee* mutation maps outside the *wee1* gene (THURIAUX, NURSE and CARTER 1978). It is very closely linked to the *cdc2* locus since no recombination was observed in 35 tetrads from crosses between this *wee* mutation and *cdc2–33*. *cdc2* mutants isolated previously were detected as temperature-sensitive conditional mutants unable to undergo mitosis at 35° (NURSE, THURIAUX and NASMYTH 1976). The *wee* mutant was originally called *wee2–1* (THURIAUX, NURSE and CARTER 1978), but since our data below are suggestive that it maps within the *cdc2* gene, we shall henceforth call it *cdc2–1w* (*w* for *wee*).

To establish the mapping relationship between *cdc2–1w* and other *cdc2* alleles, a fine-structure map of the *cdc2* locus was constructed. The levels of meiotic recombination between eight independently isolated *cdc2* mutations were measured by random spore analysis and are given in Table 3. Mutant sites *56* and *130* are probably homoallelic, as are *M26* and *M55*. Sites *L7* and *M63* were very closely linked, but are probably distinct alleles. The *cdc2* mutant sites have been organized into a fine-structure genetic map of the region shown in Figure 1. In crosses involving the *1w* mutations, there was no means of selecting for wild-type recombinants; consequently, all of the colonies arising from the cross and able to grow at 35° were examined visually. An initial screen was made for colonies that were not stained red with Phloxin B, and each of these was further examined under the microscope to determine cell length at septation. Reconstruction experiments showed that 80 to 90% of the wild-type recombinants could

TABLE 3

Recombination frequencies between cdc2 *mutant sites*

	56	*130*	*L7*	*M63*	*33*	*M26*	*M55*	*M35*	*1w*
56	<0.2	<0.2	17.7	n.t.	5.8	22.2	30.3	101.4	n.t.
					9.5	37.8	38.2	120.8	
130	<0.2	<0.2	8.0	n.t.	23.3	42.7	29.1	76.2	145
					24.9		38.8	96.5	
L7			<0.2	0.3	2.8	13.7	21.8	93.6	163
					3.8	15.1	20.0		
M63			0.9	<0.2	5.9	16.3	12.1	97.7	n.t.
					8.2		20.0	130.1	
33					<0.2	11.4	9.8	53.6	163
						13.0	17.6	66.0	
M26						<0.2	<0.2	57.1	<0.2
								74.7	
M55						<0.2	0.4	79.6	3.8
								45.9	
M35								0.5	79
1w									n.t.

Frequencies are given in wild-type recombinants/10^6 spores. The numbers of spores plated in each cross were between 5 and 15 $\times$ 10^6 except for those involving *1w*, which were between 3 and 8 $\times$ 10^5. When two frequencies are present for a particular pair of mutants, one is for the $h^- \times h^+$ cross, and the other for the $h^+ \times h^-$ cross. n.t. means not tested.

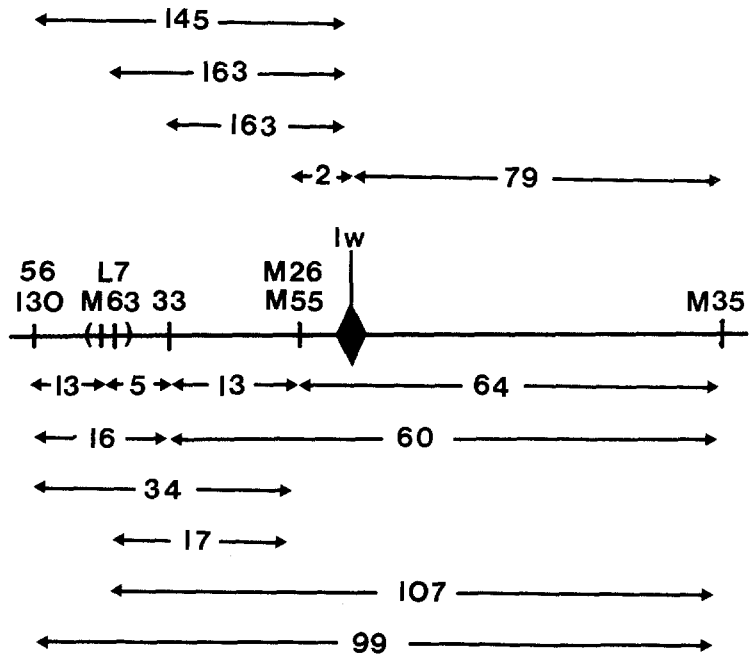

FIGURE 1.—Fine-structure map of *cdc* 2 locus. Map distances are given in fine genetic map units.

be detected by this method. The greatest distance between *1w* and any other *cdc2* mutant site was only 163 fine genetic map units, which is consistent with *1w* being located within the *cdc2* gene (Table 3). However, the precise location of *1w* is difficult to determine because of inconsistencies in the mapping data (Figure 1). *1w* was very closely linked to *M26* and *M55*, suggesting a central location within the gene. However, its levels of recombination with the other *cdc2* alleles were higher than would be expected for a central location, and favor a position to the right of *M35*. We favor the former interpretation of a central position, since the data can be understood in terms of a type of marker effect that has been observed before. Certain alleles exert marker effects in fine-structure maps in *S. pombe* that result in an expansion of map distances for alleles located some distance apart (HOFER *et al.* 1979). This phenomenon can be explained by current hybrid DNA models by a defective mismatch repair at the site of the marker-effect allele (THURIAUX *et al.* 1980). If the *1w* allele exerted a similar marker effect, then the high levels of recombination observed with the more distant *cdc2* alleles can be explained. For this reason, we have located *1w* centrally within *cdc2*, although we cannot completely rule out the possibility of a position to the right of *M35*.

These data suggest that the *cdc2* gene can mutate either to a mitosis-defective allele of the *cdc2*$^-$ type or to an allele resulting in an abnormal activity producing a *wee* phenotype, such as *cdc2–1w*. The latter suggestion is further supported by the observation that strains bearing *cdc2–56* or *cdc2–130* mutations have a reduced cell size at mitosis at the permissive temperature at 25° (Table 4). As mentioned above, these two mutations may be homoallelic. Their *wee* phenotypes at 25° segregated together wtih the *cdc2*$^-$ character in 25 tetrads tested. Furthermore, all of the 2300 *cdc2*$^+$ recombinants obtained from heteroallelic crosses between other *cdc2*$^-$ alleles and *cdc2–56* or *cdc2–130*, divided at the wild type

TABLE 4

Dominance relations of cdc2$^-$ *alleles when the mutants allele is inactive*

cdc2$^-$ allele tested	Cell length at septation in haploid strain grown at 25°		Cell length at septation in heterozygous *cdc2*$^+$/*cdc2*$^-$ diploid strain grown at 35°	
	μm	%*	μm	%†
cde2–L7	14.6	112	26.8	112
cdc2–M26	13.8	106	26.3	110
cdc2–33	14.2	109	26.5	111
cdc2–M35	22.4	172	26.6	111
cdc2–M55	13.6	105	26.1	109
cdc2–56	10.2	79	25.5	107
cdc2M63	16.7	128	25.4	106
cdc2–M72	13.4	103	25.8	108
cdc2–M76	13.6	105	26.3	110
cdc2–130	10.0	77	25.4	106

* % of wild-type haploid (13.0 μm).
† % of wild-type diploid (23.9 μm).
Strains grown in minimal medium at 25° and 35°.

size at 25°. This result argues against the possibility that *cdc2–56* and *cdc2–130* are double mutants harboring closely linked mutations that independently result in the *wee* and *cdc*⁻ phenotypes. If this had been the case, the two mutations should have been separable by recombination in the heteroallelic crosses. Therefore, a single mutation at the *cdc2* locus can result in both *wee* and *cdc*⁻ phenotypes, one phenotype being expressed at low temperature and the other at high temperature.

These results can be most easily explained if *cdc2* acts as a positive control element determining when mitosis takes place. Complete loss of *cdc2* activity prevents mitosis from taking place, resulting in the *cdc*⁻ phenotype. More subtle alterations in the *cdc2* gene product may produce abnormal activities that result in the *wee* phenotype.

Properties of wee1 *and* cdc2 *mutants—dominance relations:* To investigate further how *wee1* and *cdc2* might function in mitotic control, we examined their dominance relations. The heterozygous *wee1*⁺/*wee1–50* diploid is intermediate in cell size at division between the homozygous *wee1*⁺/*wee1*⁺ and *wee1–50*/*wee1–50* diploids, as judged by both mean protein content per cell and cell length at septation (Table 1). A similar intermediate cell length at septation was also observed for four other *wee1* alleles in *wee1*⁺/*wee1*⁻ heterozygous diploids, including the nonsense mutation *wee1–112* (Table 1). The reduction of *wee1*⁺ gene dosage from two to one results in a reduction of cell size at division of between 13 and 23%, indicating that the gene dosage of *wee1*⁺ plays some role in determining when mitosis takes place, but the effect is not large.

The diploids for investigating the dominance relations of the *cdc2* alleles fall into two classes. The first class includes diploids containing one wild-type allele and one conditional allele that prevents mitosis at the restrictive condition. Such a conditional allele is referred to as an "inactive" allele. The second class includes diploids that contain one wild-type allele and one allele that affects cell size at mitosis but does not prevent mitosis under the conditions tested. Such mutant alleles are referred to as "active" alleles. The results of the former class are shown in Table 4. Ten *cdc2*⁻ alleles were tested in heterozygous diploids with *cdc2*⁺. The cell length at division of these diploids (when grown at 35°) were all similar, being on average about 9% larger than the wild-type diploid. The recessiveness of *cdc2*⁻ alleles to *cdc2*⁺ suggests that the effect of *cdc2*⁺ on cell size at mitosis is very small since a reduction in dosage from two to one increased cell size at mitosis by only about 9%.

Dominance relations were also investigated in a number of diploids of the second class heterozygous for an "active" allele. The alleles chosen for analysis were those that result in a *wee* phenotype in haploid strains. These were *cdc2–1w* grown at 35°, and *cdc2–56* and *2–130* grown at 25° (Table 5). All three heterozygous diploids were intermediate in cell size at division, but were closer in size to the respective homozygous mutant diploids than to the wild-type diploid (Table 5). The increases in cell size of the heterozygous diploids over the respective homozygous diploids were only between 5 and 13%. Therefore, active *cdc2* alleles that cause a *wee* phenotype are almost completely dominant over *cdc2*⁺,

TABLE 5

Dominance relations of cdc2 *alleles when the mutant allele is active*

Strain	Temperature of growth	Cell length at septation µm	Cell length at septation %*	Mean protein content per cell pg/cell	Mean protein content per cell %*
cdc2+/cdc2+	35°	23.9	100	22.6	100
cdc2+/cdc2-1w	35°	15.6	65	15.8	70
cdc2-1w/cdc2-1w	35°	13.4	56	12.8	57
cdc2+/cdc2+	25°	20.3	100	—	—
cdc2+/cdc2-56	25°	16.6	82	—	—
cdc2+/cdc2-130	25°	16.9	83	—	—
cdc2-56/cdc2-56	25°	15.2	75	—	—
cdc2-130/cdc2-130	25°	15.8	78	—	—

* % of wild-type diploid *cdc2+/cdc2+* grown at 25° or 35°.
Strains grown at 25° and 35° in minimal medium.

further supporting the suggestion that the *wee* mutant phenotype is the result of an abnormal activity of the *cdc2* gene produce.

DISCUSSION

The simplest interpretation of our data is that the *wee1+* gene codes for a negative element or inhibitor in the mitotic control, and that its dosage plays some role in determining when mitosis takes place. In contrast, the *cdc2+* gene codes for a positive element or activator, and its dosage has almost no effect on the control. In addition, the *wee* mutant alleles at the *cdc2* locus demonstrate that some aspect of the *cdc2* gene product activity is important for the timing of mitosis.

The properties of the *cdc2* gene are intriguing, since the mitotic control is influenced by the *cdc2* gene product activity, but not by the *cdc2* gene dosage. This is unexpected because changes in gene dosage are likely to alter the total amount of gene product synthesized, which should also change the total gene product activity within the cell. Two types of hypotheses can account for these properties. The first is that the *cdc2* regulatory control circuit can compensate for changes in gene dosage. One possible circuit is an autogenous feedback loop, whereby the *cdc2* gene product regulates its own synthesis and maintains itself at a constant concentration. If gene dosage is reduced, as in the heterozygous *cdc2+/cdc2-* diploid, synthesis from the single active *cdc2+* gene is increased so that the concentration of the *cdc2* gene product is maintained at the same level found in the wild-type *cdc2+/cdc2+* diploid. The second type of hypothesis is that the *cdc2* gene is not dosage compensated. Some *cdc2+* gene product must be present before mitosis can take place, but the timing of mitosis is not influenced by the total amount of gene product present in the cell. The timing of mitosis is determined by a particular aspect of the *cdc2* gene activity, such as an allosteric interaction with another component of the control system, and this aspect of the activity is altered in the *wee* mutant alleles.

The *wee1*$^+$ and *cdc2*$^+$ genes influence the timing of mitosis during the cell cycle. Two other parameters that also influence the timing of mitosis are cell size and cell growth rate. Cells have to attain a certain size before undergoing mitosis. Cells growing in steady state tend to undergo mitosis and cell division at a characteristic size, and a homeostatic mechanism that maintains that size (FANTES 1977) operates at the control of mitosis (FANTES and NURSE 1978). The growth rate or nutritional status of a cell is also important for mitotic control. This has been shown by transferring cells from poor to rich medium, and *vice versa*, and observing a corresponding transient acceleration or inhibition of the rate at which wild-type cells enter mitosis (FANTES and NURSE 1977). This growth rate or nutritional status modulation of the mitotic control is not observed with *wee1* (FANTES and NURSE 1978). Mitosis takes place when the cell attains a certain size, but this size can be modulated up or down according to the growth rate or nutritional status of the cell.

Given these effects of cell size and growth rate on the mitotic control, one possible role for the *wee1*$^+$ and *cdc2*$^+$ gene products is that they function in the mechanisms monitoring these parameters. Various models for monitoring cell mass have been proposed that make certain predictions about gene dosage effects on cell size at division (FANTES *et al.* 1975). None of these models predict the precise gene dosage behavior of the *wee1* and *cdc2* genes, although it is of interest that the initiator protein of the autorepressor model of SOMPAYRAC and MAALØE (1973) is a positive element, and its rate of expression is gene-dosage compensated. Another role for the *wee1*$^+$ and *cdc2*$^+$ gene products is that they may be involved in the monitoring of cell growth rate or nutritional status (FANTES and NURSE 1978). Because of these mutations, *wee1*$^-$ and *cdc2–1w* cells may permanently consider themselves to be in poor medium with a low growth rate and poor nutritional status. As a consequence, they undergo mitosis and cell division at the small size characteristic of growth in poor medium. This general control monitoring growth rate or nutritional status may be analogous to other general metabolic controls, such as the relaxed-stringent control in bacteria (CASHEL and GALLANT 1974) and possibly in fungi (STURANI, MAGNANI and ALBERGHINA 1973; OLIVER and MCLAUGHLIN 1977), the pleiotypic control in mammalian cells (HERSHKO *et al.* 1971) or the general control of biosynthetic pathways found in *S. cerevisiae* (WOLFNER *et al.* 1975). If this is so, then there may also be differences in general metabolism in strains bearing lesions in the *wee1* and *cdc2* genes, leading to the exciting possibility that these two genes are involved in a general control monitoring the rate of metabolism, one of the functions of which is to regulate the timing of mitosis.

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