Regulation of *Schizosaccharomyces pombe* Wee1 Tyrosine Kinase*

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Wee1 tyrosine kinase regulates mitosis by carrying out the inhibitory tyrosine 15 phosphorylation of Cdc2 M-phase inducing kinase. *Schizosaccharomyces pombe* Wee1 is a large protein, consisting of a C-terminal catalytic domain of ~350 amino acids preceded by a N-terminal domain of ~550 residues. The functional properties of the Wee1 N-terminal domain were investigated by expressing truncated forms of Wee1 in *S. pombe*. Both positive and negative regulatory domains were identified. Sequences important for Wee1 function were mapped to a central region (residues 363–408). This region is not required for kinase activity or nuclear localization, suggesting it may be involved in substrate recognition. The negative regulatory domain resides in the N-terminal third of Wee1. Wee1 constructs lacking this domain are more effective at delaying mitosis than wild-type Wee1. The negative regulatory domain contains clusters of potential Cdc2 phosphorylation sites. Investigations to monitor the abundance of Wee1 mRNA and protein during the cell cycle were also carried out.

A key aim of cell cycle investigations has been to fully describe the control mechanism that regulates the onset of mitosis, which is often referred to as the mitotic control. In recent years major advances have been made in understanding the mitotic control, largely as a result of the confluence of several experimental approaches, in particular genetic and *in vivo* biochemical studies carried out with the fission yeast *Schizosaccharomyces pombe* and *in vitro* biochemical studies carried out with extracts made from oocytes of the frog *Xenopus laevis* (1). These studies have led to the following understanding of the mitotic control. The initiation of mitosis is brought about by Cdc2 serine/threonine kinase, acting in obligatory association with one or more B-type cyclins. B-type cyclins that are involved in promoting mitosis are periodically destroyed upon exit from M-phase, thus progression through S and G2 is accompanied by a steady increase in the abundance of Cdc2-cyclin B complex. Activation of Cdc2 also requires phosphorylation of a threonine residue in the T-loop region (threonine 167 in *S. pombe* Cdc2), although this phosphorylation does not appear to have an important role in regulating the periodic activation of Cdc2 (2). Cdc2-cyclin B is maintained in a repressed state during interphase due to phosphorylation of a tyrosine residue in the N-terminal lobe of Cdc2 (3). In *S. pombe* phosphorylation of Cdc2 on tyrosine 15 is performed by Wee1 and Mik1 tyrosine kinases, with Wee1 having the dominant role (4–11). In animal cells this phosphorylation is carried out by Wee1 and Myt1 kinases, with the latter enzyme also phosphorylating the preceding threonine residue (7, 12–17). Dephosphorylation of these residues and consequent activation of Cdc2 is largely carried out by Cdc25 dual specificity phosphatase (18–24), although other phosphatases may weakly contribute to the activation of Cdc2-cyclin B (25, 26).

Wee1 and Cdc25, two of the major regulators of Cdc2-cyclin B kinase, are themselves regulated by phosphorylation (1). In *S. pombe*, Nim1 serine/threonine protein kinase contributes to the induction of mitosis by carrying out inhibitory phosphorylation of Wee1 (27–31). In *Xenopus* oocytes and human tissue culture cells, Wee1 is the subject of a separate form of inhibitory phosphorylation that occurs during M-phase (15, 32–34). This second mechanism of inhibitory regulation of Wee1 is either directly or indirectly controlled by Cdc2-cyclin B kinase. Cdc25 phosphatase undergoes activating phosphorylation during M-phase, also by a mechanism that is either directly or indirectly controlled by Cdc2 (35–43). This type of Cdc25 regulation has been demonstrated in mammalian tissue culture cell, *Xenopus* oocyte extracts, and *S. pombe*.

It has been proposed that the activating phosphorylation of Cdc25 and inhibitory phosphorylation of Wee1 that occurs during M-phase may play an important part in promoting the onset of mitosis (1). In this positive feedback model, activation of a small fraction of Cdc2-cyclin B can be rapidly amplified into total activation by a process in which Cdc2-Cyclin B catalyzes stimulatory phosphorylation of Cdc25 and inhibitory phosphorylation of Wee1 (1). The rod-shaped fission yeast presents one of the best experimental systems to test this positive feedback model of mitotic control. In *S. pombe* the size at which cells initiate mitosis and undergo cell division is quite sensitive to the gene dose of *wee1*+, *cdc25*+, and *nim1*+, thus determination of cell length at division provides an easy measurement of mitotic timing (4, 5, 23, 30). In rich growth medium wild-type cells divide at ~15 μm in length, *wee1*− cells undergo division at approximately half the length of wild-type and exhibit a whee phenotype, whereas cells that have extra copies of *wee1*− undergo division at longer cell lengths that are directly related to *wee1*− gene dose (5).

This report deals with the function and regulation of Wee1 in *S. pombe*. Fission yeast Wee1 is a large protein, having a molecular mass of ~107 kDa (5). The protein kinase catalytic domain is confined to the C-terminal ~35 kDa of Wee1. Interestingly, the protein sequences of the large N-terminal domains of Wee1 homologs from *S. pombe*, the budding yeast *Saccharomyces cerevisiae*, the fruit fly *Drosophila melanogaster*, *Xenopus*, and humans are highly divergent (5, 15, 34, 44, 45). For this reason very little is known about which regions of Wee1 other than the catalytic domain are required for function *in vivo*. *In vitro* studies have shown that a truncated form of Wee1 containing only the ~35-kDa catalytic domain, produced in insect cells, is unable to phosphorylate Cdc2, although it is able to phosphorylate enolase, suggesting that some part of the...
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N-terminal domain is important for substrate interaction (8). One feature that appears to be shared among Wee1 proteins from different species is a preponderance of serine-proline and threonine-proline dipeptides. SP and TP are the minimal consensus sequences for phosphorylation catalyzed by Cdc2 (46), thus one attractive model is that Cdc2 or another proline-directed protein kinase promotes the initiation of mitosis by carrying out inhibitory phosphorylation of Wee1. A key prediction of this model is that elimination of phosphorylation sites, either by truncation or mutation, should make Wee1 resistant to inhibition by phosphorylation and as a consequence the initiation of mitosis should be delayed or prevented.

EXPERIMENTAL PROCEDURES

Yeast Strains and Media, Genetic Methods, and Cell Length Measurements—S. pombe strains are all derived from 972h- and 975h- (47). Procedures for genetic studies in S. pombe have been described (48). YES and synthetic EM2 media were used to grow S. pombe cells (49). Cell size measurements were determined using an eyepiece micrometer attached to a Zeiss Axiokop 20 microscope with a 100 X objective.

Wee1 Truncation and Epitope Tag Constructs—The wee1 open reading frame was amplified by PCR from pWEE1–1 (5) using the 5’ oligonucleotide 5’-CTCATATGAGATCTTATGGCTTTACGGCGGTCC-3’ (NcoI site underlined) and the 3’ oligonucleotide 5’-CTCATATGAGATCTTATGGCTTTACGGCGGTCC-3’ (NdeI site underlined). The underlined sequence is the sequence, GTGGATTGTTGGCGGCCCATCTTT-ACCCATACATGTTCTCAGATCGGGCTATCTAAGGC.

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kinases. A series of wee1 constructs encoding proteins having N-terminal truncations or internal deletions were produced (see “Experimental Procedures”) and placed under the control of the thiamine (vitamin B₁₂)-repressible nmt1 promoter (49). Plasmids containing these constructs were integrated at the wee1 locus in wild-type and wee1–50 mik1::ura4 strains, in each case leaving the genomic copy of wee1⁺ or wee1–50 intact. Note that wee1–50 is a temperature-sensitive mutation, thus wee1–50 mik1::ura4 cells are viable and only slightly smaller than wild-type at the permissive temperature of 25 °C, but undergo mitotic catastrophe when incubated at temperatures above 30 °C (11). Southern hybridization analysis confirmed that these strains contained a single integrated copy of the plasmids. At the onset we noticed that the full-length version of nmt1::wee1⁺ caused a moderate cell elongation phenotype in the wee1–50 Δmik1 background when these cells grown in medium that represses the nmt1 promoter (Fig. 1). Septated cells were ~21 μm in length, versus ~15 μm for wild-type cells. This suggests that the repressed nmt1⁺ promoter is several-fold more active than the wee1⁺ promoter, underscoring the fact that wee1⁺ expression is normally quite low.

Most of the wee1 constructs caused cell cycle arrest when expressed from the induced nmt1 promoter in the wild-type and wee1–50 Δmik1 backgrounds. These included the wee1–32 construct, which lacked the N-terminal 310 amino acids, as well as the wee1–37 construct, which lacked the N-terminal 310 and internal 408–520 amino acids (Fig. 1). Constructs which failed to cause a cell cycle arrest when highly overexpressed included wee1–3, which lacked the N-terminal 460 amino acids, wee1–5, which lacked 520 amino acids, and wee1–9, which lacked the internal 310–460 amino acids. The failure of constructs such as wee1–3 to cause cell cycle arrest could not be attributed to a problem with protein expression; in fact, the amount of Wee1 protein detected in cells expressing inactive constructs such as wee1–3 and wee1–5, was much higher than in cells expressing active constructs such as wee1–3 and wee1–5, which in turn expressed higher levels of Wee1 protein than cells expressing full-length wee1⁺ from the nmt1 promoter. It appears that cells expressing active wee1 constructs experience growth defects caused by a massive increase in size long before the nmt1 promoter is fully derepressed, this may explain why inactive wee1 constructs appear to be more abundant that active wee1 constructs. It is also possible the shorter Wee1 constructs are more stable proteins. Nor could the properties of inactive constructs be attributed to loss of kinase activity per se, as the minimal catalytic domain of Wee1 (residues 520–877, equivalent to the catalytic domain of Wee1 (residues 520–877, equivalent to wee1–50 mik1::ura4, when produced in an insect cell expression system, retains vigorous autophosphorylation activity (8)). This finding was confirmed for the wee1–3 and wee1–5 constructs expressed in S. pombe, which also retained vigorous autophosphorylation activity. It should be noted that high overexpression of the only wee1–3, wee1–5, and wee1–9 constructs caused a moderate elongation of cell length at division in wild-type cells and these constructs were able to rescue wee1–50 Δmik1 (Fig. 1), indicating that these truncated forms of Wee1 were not completely inactive in vivo.

The inability of the wee1–9 construct to cause cell cycle arrest when expressed from the nmt1 promoter strongly suggested that amino acids 310–460 are critical for Wee1 function in vivo. Indeed, construct wee1–6, which consists of the 310–460 region fused to the catalytic domain (residues 520–881), was able to cause cell cycle arrest when overexpressed from the nmt1 promoter (Fig. 1). The important sequences in the 310–460 region were further delineated by testing the activity of the wee1–6 Δmik1 construct, which contained amino acids 310–408 fused to the catalytic domain (Fig. 1). This construct also was

FIG. 1. Map of nmt1::wee1 truncation constructs and table of phenotypes. The linear map of the 881-amino acid Wee1 polypeptide and extent of various truncation and/or deletions is shown at the right. Numbers refer to amino positions that correspond to various truncation and internal deletion positions. Protein kinase domain (gray area) refers to the region having homology to catalytic regions of serine/threonine protein kinases. Black area indicates 363–408 region that when fused to the catalytic domain (construct wee1–Δ8) restores ability to cause cdc arrest when overproduced from nmt1 promoter and to rescue wee1–50 Δmik1 mitotic catastrophe when grown in nmt1 derepressing medium. Hatched area indicates the region that when deleted (construct wee1–Δ9) severely compromises the ability of Wee1 protein to inhibit mitosis. Constructs were integrated at single copy into wild-type and wee1–50 Δmik1 backgrounds. Phenotypes were evaluated in nmt1 derepressing conditions (“on”) and repressing conditions (“off”). Integrants in the wild-type background were grown at 30 °C, integrants in the wee1–50 Δmik1 background were grown at 35.5 °C. Phenotypes were scored and classified into the following categories: approximately wild-type in length (mc), approximately wild-type in cell division arrest (mc), and approximately wild-type in cell division arrest (mc). Cells overproduced from nmt1 promoter and wee1–50 Δmik1 were grown in derepressing medium (Hatched). Cells overproduced from nmt1 promoter were grown at 30 °C, integrants in the wild-type background were grown at 30 °C, integrants in the wee1–50 Δmik1 background were grown at 35.5 °C. Phenotypes were scored and classified into the following categories: approximately wild-type in length (mc) at division, approximately 50% longer than wild-type (m) at division, approximately 100% longer than wild-type (m) at division, cells underwent division at an extremely small size and exhibited a typical mitotic catastrophe phenotype (m) as described previously (11, 23), strain not constructed and phenotype not determined (n.d.).

* R. Aligue and P. Russell, unpublished observations.
active, causing cell cycle arrest when overexpressed from the nmt1 promoter and rescuing wee1–50 Δmik1 when cells were grown in nmt1 repressing medium. We finally tested the activity of construct wee1-Δ8, which contained amino acids 363–408 fused to C-terminal protein kinase domain. This construct also caused cell cycle arrest when expressed from the nmt1 promoter and rescued wee1–50 Δmik1 when cells were grown in nmt1 repressing medium (Fig. 1). These findings indicate that N-terminal sequences that are critical for Wee1 function are located in the 363–408 amino acid region.

The Critical Central Region of Wee1 Is Not Required for Proper Localization of Wee1 Protein—Cdc2-Cdc13 complex is predominantly and perhaps exclusively localized in the nucleus during G2 (52–54). We therefore expected that Wee1 would also be a nuclear protein. This was investigated by carrying out immunolocalization studies of Wee1. Anti-Wee1 antibodies produced no signal in wild-type cells, presumably because of the very low abundance of Wee1 protein. To circumvent this problem we overexpressed wee1-K596L, which encodes a catalytically inactive form of Wee1 (6, 55). Staining with anti-Wee1 antibodies produced a strong signal that closely coincided with the nuclear DNA signal produced by staining with DAPI (Fig. 3, top panels). The nmt1:wee1-K596L construct was expressed from an episomal plasmid that was frequently lost during mitotic divisions, therefore only a subset of cells stained with the anti-Wee1 antibody. These observations indicate that Wee1 protein is localized in the nucleus, a finding consistent with immunolocalization studies of overexpressed Wee1 protein encoded by wee1–50 (56).

The critical central region of Wee1 contains a stretch of basic amino acids 387-LSKQHRPRKNT-397 (basic residues underlined) that potentially could be involved in directing the nuclear localization of Wee1 (57). This possibility was initially supported by the observation that Wee1-Δ4 protein, which consists only of the catalytic domain of Wee1, was predominantly localized in the cytoplasm (Fig. 3, middle panels). Immunolocalization analysis of Wee1-Δ9 protein, which specifically lacks the critical central region together with some flanking sequence (Fig. 3, bottom panels), provided a more direct test of this hypothesis. This analysis showed that Wee1-Δ9 protein was predominantly localized to the nucleus, producing a signal that was very similar to that observed with Wee1-K596L. These findings suggest that the 363–408 amino acid region is not essential for nuclear localization, although it may be capable of promoting nuclear localization in vivo.

The Region Comprising the N-terminal ~300 Amino Acids of Wee1 Has a Negative Effect on Total Wee1 Activity in Vivo—The nmt1:wee1 truncation and deletion constructs divided into four classes when assayed for their ability to rescue wee1–50 Δmik1 in nmt1-repressing medium (Fig. 1). The wee1-Δ3 and wee1-Δ4 constructs failed to rescue wee1–50 Δmik1, constructs wee1-Δ6, -Δ7, and -Δ8 rescued but did not cause an elongated phenotype; full-length wee1+ caused cells to divide at ~21 μm, whereas three of the constructs having an N-terminal deletion (i.e. wee1-Δ1, -Δ2, and -Δ5) caused cell elongation phenotype that was more severe than that caused by full-length wee1+. The phenotypes caused by the wee1-Δ1, -Δ2, and -Δ5 constructs suggested that the N-terminal domain of Wee1 might have an inhibitory effect on Wee1 activity. This was more carefully investigated by integrating a subset of wee1 truncation constructs, expressed from the wee1+ promoter, into a wee1–50 Δmik1 background. Integrants were analyzed by Southern hybridization to confirm that they have a single copy of the wee1 truncation constructs. The activity of the constructs was first assayed by determining whether they rescued the mitotic catastrophe phenotype observed in wee1–50 Δmik1 cells incubated at the restrictive temperature of 35.5 °C. The wee1-Δ4 construct failed to rescue the mitotic catastrophe phenotype, whereas the wee1-Δ1, wee1-Δ2, and wee1-Δ7 constructs complemented the defect (Table I). These findings are consistent with the behavior of the nmt1:wee1 constructs as described in Fig. 1. Cell size measurements were then carried out with cultures grown at 25 °C. This analysis showed that cells having single integrated copies of wee1-Δ1, wee1-Δ2, or wee1-Δ7 were significantly longer at division (22.4 ± 1.0, 23.9 ± 1.2, and 21.6 ± 0.9 μm, respectively), than were cells having a single integrated copy of wee1+ (16.8 ± 2.4 μm). On average the cells expressing the activated truncation alleles of wee1 were 35% larger than...
cells expressing wee1<sup>+</sup>. These findings show that truncation of the N-terminal ~300 amino acids of Wee1 results in a greater amount of Wee1 activity in vivo. This may be due either to increased specific activity of Wee1 or to greater stability of Wee1 protein.

Measurements of the Abundance of wee1<sup>+</sup> mRNA and Wee1 Protein during the Cell Cycle—The timing of mitosis is very sensitive to the gene dose of wee1<sup>+</sup>, therefore periodic changes in the cellular concentration of Wee1 would be predicted to affect mitotic timing. For this reason a secondary aim of our studies was to determine whether the amount of wee1 mRNA or Wee1 protein change during the cell cycle. We first measured wee1 mRNA expression during the cell cycle. A synchronous culture of wild-type cells was prepared by centrifugal elutriation. This method separates cells on the basis of size, generating a pure population of small cells that are in early G<sub>2</sub> (58). These cells were cultured for two cell cycles. At regular intervals samples were collected for RNA preparation and Northern blot analysis. Cell cycle periodicity was monitored by counting the septation index. In agreement with previous studies (5), two wee1 specific mRNA transcripts were detected, a more prominent ~4.0-kb species and a less abundant ~3.4-kb form (Fig. 4). As expected, these mRNA species were absent in a strain in which the wee1<sup>+</sup> open reading frame was replaced with the ura<sup>4</sup> gene (wee1::ura<sup>4</sup>). As shown in Fig. 4, the level of wee1<sup>+</sup> mRNA did not appear to fluctuate during two synchronous rounds of division.

Experiments designed to monitor the abundance of Wee1 protein during the cell cycle were problematic because of the extreme paucity of Wee1 protein. Wild-type levels of Wee1 could not be reliably detected by standard immunoblotting methods. Wee1 is a dose-dependent inhibitor of mitosis, therefore the Wee1 detection problem could not be solved by increasing wee1<sup>+</sup> copy number. We initially solved this problem by creating a strain in which the genome copy of wee1<sup>+</sup> was replaced with a version of wee1<sup>+</sup> that encoded Wee1 protein having an C-terminal extension consisting of three copies of the HA epitope, which is recognized by 12CA5 monoclonal antibodies (anti-HA). Cell size was not noticeably affected by the replacement of wild-type wee1<sup>+</sup> with the wee1<sup>+</sup>-3HA allele. A synchronous culture of the wee1<sup>+</sup>-3HA strain was made by centrifugal elutriation. Immunoblot analysis showed that a Wee1 signal was present throughout the cell cycle, but it appeared to undergo a moderate oscillation (Fig. 5). The Wee1 signal was highest in samples 1–3 and 7–11, these samples correspond to S and G<sub>2</sub> phases. In the first cell cycle the initial decrease in the Wee1 signal, occurring in samples 3–4, immediately preceded the rise in septation index. Septation follows the onset of mitosis in S. pombe, thus the decrease in the Wee1 signal closely corresponds to M and G<sub>1</sub> phases.

**TABLE I**

<table>
<thead>
<tr>
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<th>25 °C</th>
<th>35 °C</th>
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<tbody>
<tr>
<td>Control</td>
<td>16.1 ± 1.2 μm</td>
<td>Mitotic catastrophe</td>
</tr>
<tr>
<td>Wee1&lt;sup&gt;+&lt;/sup&gt;</td>
<td>16.8 ± 2.4 μm</td>
<td>Viable</td>
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<tr>
<td>Wee1-Δ1</td>
<td>22.4 ± 1.0 μm</td>
<td>Viable</td>
</tr>
<tr>
<td>Wee1-Δ2</td>
<td>23.9 ± 1.2 μm</td>
<td>Viable</td>
</tr>
<tr>
<td>Wee1-Δ4</td>
<td>15.9 ± 0.9 μm</td>
<td>Mitotic catastrophe</td>
</tr>
<tr>
<td>Wee1-Δ7</td>
<td>21.5 ± 0.9 μm</td>
<td>Viable</td>
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**FIG. 4.** Northern blot analysis of wee1 mRNA abundance during the cell cycle. A synchronous culture of wild-type cells (PR109) was produced by centrifugal elutriation; cell cycle synchrony was monitored by counting the septation index (lower panel). The Northern blot was probed with the 5-kb EcoRI/XhoI DNA fragment covering the wee1 open reading frame and ~1.5 kb of downstream flanking sequence (upper panel). Lane 0 contains RNA from a wee1::ura<sup>4</sup> strain, showing that ~4.0-kb mRNA and a less abundant ~3.4-kb species are derived from wee1. A smaller mRNA species from an adjacent gene was detected with the probe and thus served as one loading control; the Northern blot was also probed with adh1 probe to confirming equal loading. The abundance of wee1 mRNA species appeared constant during the cell cycle.

**FIG. 5.** Immunoblot analysis of Wee1 protein during the cell cycle. This experiment used a strain (LW1685) in which the genomic wee1<sup>+</sup> encoded Wee1 protein having three copies of the HA epitope at the C terminus of the protein. A synchronous culture of these cells was produced by centrifugal elutriation; cell cycle synchrony was monitored by counting the septation index (lower panel). The immunoblot was probed with anti-HA monoclonal antibody 12CA5 (top panel) and anti-Cdc2 polyclonal antibody (middle panel). Lane 0 contains protein extract from a wild-type strain which produces untagged Wee1. The Wee1 signal appeared to oscillate during the two cell cycles, being lowest in the samples corresponding to M and/or G<sub>1</sub>.

**DISCUSSION**

In this study we have focused on several key issues relevant to the structure and function of S. pombe Wee1 tyrosine kinase. One aim of our studies was to identify regions of the Wee1 N-terminal noncatalytic domain that are important for Wee1 function in vivo. As noted above, there are no obvious sequence homologies between N-terminal domains of Wee1-like proteins isolated from divergent species, therefore it was unclear which sequences of the N-terminal domain would have functional importance. In fact, our studies indicate that only a small region of the N-terminal domain is of critical importance. By testing various N-terminal truncations and internal deletions the critical region was initially localized to amino acids 310–460. We proceeded to show that the wee1-Δ8 construct, containing only residues 363–408 fused to the catalytic domain (residues 520–881), retained the ability to cause cdc arrest when overexpressed from the derepressed nmt1 promoter. Moreover, wee1-Δ8 also rescued wee1<sup>-50</sup> Δmik1 mitotic catastrophe when expressed from the repressed nmt1 promoter.

What function is provided by the 363–408 region of Wee1? Previous studies showed that a purified ~37-kDa C-terminal
construct of Wee1 was able to phosphorylate enolase but not Cdc2-cyclin B in vitro (8). In contrast, purified full-length Wee1 phosphorylated Cdc2 (when complexed to cyclin B) but did not phosphorylate enolase (8). These results strongly suggest that the Wee1 N-terminal regulatory sequences are not required for Wee1 protein kinase activity per se. Our in vivo studies extend these findings by showing that the wee1Δ4 construct, which encodes the same protein as the aforementioned ~37-kDa Wee1 truncation, is extremely defective at inhibiting mitosis in vivo. Fusion of the 363–408 region to ~37 kDa Wee1 restores in vivo mitotic inhibition activity to a level that is near wild-type levels. It is likely that the 363–408 sequence is important for substrate recognition. In this regard it is worth recalling that previous in vitro studies showed that monomeric Cdc2 is a very poor substrate for Wee1, whereas Wee1 readily phosphorylates Cdc2 that is bound to cyclin B (8). This raises the question of whether cyclin-B forms all or part of the structure recognized by Wee1, or whether the primary affect of cyclin B is to induce a conformational change in the structure of Cdc2 such that the region encompassing tyrosine 15 is exposed to Wee1 (and Cdc25).

A second major aim of our studies was to determine whether the N-terminal region of Wee1 contains domains of inhibitory regulation and to evaluate the affect of deleting those domains on the mitotic control. We found that truncation of amino acids 1–153 or 1–310 (wee1Δ1 and wee1Δ2) led to a delay of the onset of mitosis, as shown by an increase of the length of cells at division. This was observed both for cells having nmt1::wee1 constructs that were grown in nmt1 repressing conditions, as well as for cells having single integrated copies of the wee1 constructions expressed from the wee1 promoter. The observed cell elongation is roughly equivalent to that observed in cells carrying 2–3 extra copies of wee1Δ1 integrated at the wee1 locus (5). Thus we may surmise that by truncating Wee1 we have interfered with the mechanism of mitotic timing. None of the constructs caused a cell cycle arrest when expressed from the wee1Δ1 promoter at single copy. This may indicate that negative regulation of Wee1 mediated through the N-terminal domain has a modulatory effect but is not essential for the induction of mitosis. As noted above, our findings do not necessarily imply that the specific activity of Wee1 is increased by truncation of the N-terminal region of Wee1, since it is also possible that the truncation stabilizes Wee1 protein, leading to an increased level of Wee1 kinase in vivo.

A third aim of our studies was to provide some basic information about the regulation of wee1 mRNA and protein expression during the cell cycle. We found that the wee1Δ1 mRNA Northern blot signal did not fluctuate during the cell cycle, whereas the Wee1 protein immunoblot signal did undergo a moderate oscillation. The decrease in the Wee1 immunoblot signal appeared to coincide with M-phase, thus our data suggest that Wee1 might be degraded during this phase of the cell cycle. Experiments are currently underway to determine whether Wee1 is destabilized in cells that are arrested in M-phase. Our data do not suggest that Wee1 abundance changes prior to the onset of mitosis, so we do not believe that oscillation of Wee1 protein plays a role in determining the timing of mitosis.

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