These results implicate events during the preceding winter, namely intraspecific competition for optimal winter habitat mediated through behavioral dominance (*8, 20*), as an important factor determining arrival times and condition upon arrival of redstarts in their north temperate breeding areas. This finding is important because arrival time at the breeding ground is a major determinant of fitness in migratory birds (*3, 21*). Furthermore, our evidence that later arriving birds wintered in drier habitats and that physical condition declined with arrival date suggests that optimal winter habitats for redstarts may be saturated and therefore limiting. If optimal winter habitats (more mesic sites) were always available, then all redstarts should have occupied them, and we would have found no relation between $\delta^{13}C$ values and the physical condition of redstarts over the arrival period. This conclusion, that winter habitats are limiting, has important conservation implications for the long-term stability of migratory bird populations, many of which are declining and of conservation concern (*22*).

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15. The $\delta^{13}C$ values in insects collected from within the foraging height range of redstarts in two of the habitats in Jamaica differed significantly [$F(1,10) = 15.24$, $P = 0.004$], with $\delta^{13}C$ values of forest insects being more depleted [-25.8 ± 0.44 per mil (mean ± SE)] and insects from scrub territories more enriched (-24.2 ± 0.20 per mil).
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17. Blood tissue samples were collected from redstarts in Jamaica and Honduras, and pectoral muscle tissue was collected from redstarts during the arrival period in New Hampshire. Both types of tissue equally reflect dietary integrations over the previous 6 to 8 weeks (*20*). All samples were freeze-dried and powdered in a dental amalgam mill. About 1 mg of sample was combusted in a Robo-Prep elemental analyzer interfaced with a 20:20 Europa continuous-flow stable-isotope mass spectrometer. Two reference standards were run for every five unknowns, and on the basis of replicate measurements of an egg albumen standard, we estimate our analytical precision to be ±0.1 per mil. All stable-isotope values are reported in δ notation relative to the Pee Dee Belmnite standard: $\delta^{13}C = 1000 \times \{[(^{13}C_{unk}/^{12}C_{unk}) \div (^{13}C_{std}/^{12}C_{std})] - 1\}$. We found no difference in $\delta^{13}C$ values of blood, muscle, and insect samples that were either treated with a 0.5 N HCl solution to remove carbonates or lipid extracted with a chloroform: methanol rinse, so we report here only those values based on replicate measurements of untreated blood, muscle, or insects.
18. The $\delta^{13}C$ values of redstarts arriving in breeding areas should reflect mostly those of habitats occupied on the wintering grounds for several reasons. Although food intake during migration may influence $\delta^{13}C$ values, muscle tissue values have been shown to reflect dietary integrations over the previous 6 to 8 weeks [K. A. Hobson and R. G. Clark, *Condor* **94**, 181 (1992)]. Furthermore, energy demand during migration primarily involves metabolism of fat rather than muscle protein [M. Ramenofsky, in *Bird Migration: Physiology and Ecophysiology*, E. Gwinner, Ed. (Springer-Verlag, Berlin, 1990), pp. 214–231], thus turnover of protein is low. Even if redstarts wintering in scrub habitats had moved into wetter habitats once dominant redstarts left, their muscle $\delta^{13}C$ values would begin to become depleted, not enriched as was found.
19. By controlling for the structural size of individuals and relating this to body mass, it is possible to assess the physical condition of birds. Redstarts with a body mass light for their structural size were considered in poor physical condition relative to those heavy for their structural size. To calculate mass corrected for structural size, we calculated the scores of a principal component analysis on the basis of wing chord, tarsus, and bill length, and then regressed body mass against these scores. The residuals from this regression estimate mass corrected for structural size and provide our index of physical condition.
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Coupling of Mitosis to the Completion of S Phase Through Cdc34-Mediated Degradation of Wee1

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The dependence of mitosis on the completion of the period of DNA replication in the cell cycle [synthesis (S) phase] ensures that chromosome segregation occurs only after the genome has been fully duplicated. A key negative regulator of mitosis, the protein kinase Wee1, was degraded in a Cdc34-dependent fashion in *Xenopus* egg extracts. This proteolysis event was required for a timely entrance into mitosis and was inhibited when DNA replication was blocked. Therefore, the DNA replication checkpoint can prevent mitosis by suppressing the proteolysis of Wee1 during S phase.

Dividing cells depend on ubiquitin-mediated protein destruction for proper cell cycle progression. At least three distinct cell cycle transitions are regulated by proteolysis: passage from the prereplicative phase of growth (G_1) to S phase, passage through metaphase, and exit from mitosis (*1*). Exit from mitosis depends on the proteolysis of the cyclin subunit of the maturation promoting factor (MPF); this is accomplished by the anaphase promoting complex (APC)/cyclosome, a large multi-subunit complex that functions as a ubiquitin ligase. The APC also regulates entrance into anaphase by promoting the separation of sister chromatids that is due to the destruction of the Pds1 (budding yeast) and Cut2 (fission yeast) proteins (*1*). In yeast, the Sic1 protein, an inhibitor of the S phase cyclin-dependent kinase that initiates DNA replication, is degraded to

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allow entrance into S phase (*2*). Degradation of Sic1p is accomplished by the Cdc34 ubiquitin-conjugating enzyme in combination with the Skp1/Cdc53/F-box protein (SCF) complex, which contains the Skp1, Cdc4, and Cdc53 gene products (*2*). The inactivation of Cdc34 or of any of the SCF subunits blocks Sic1p degradation and arrests the cell cycle at the transition point between the G_1 and S phases.

Other cell cycle transitions may also require SCF-mediated protein degradation. Genetic experiments in yeast have shown that cells harboring certain mutant alleles of Skp1 (*3*) or Cdc34 (*4*) arrest as large budded cells with a single nucleus, suggesting a role for these proteins at the transition from G_2 to M. We used cycling extracts derived from *Xenopus* eggs to assess the effects of the inhibitors of ubiquitin-mediated proteolysis on the transition from S phase into mitosis. The in vitro cell cycle was initiated by combining cycling extracts with sperm nuclei. Because proteolysis inhibitors block the initiation of DNA replication if they are added before the addition of sperm nuclei [but not if they are added after initiation is complete (*5*, *6*)], we allowed nuclear assembly and the initiation of replication to occur for 20 min before the addition of exogenous reaction components. This ensured that complete DNA replication occurred and that any G_2 arrest detected did not result from the activation of the DNA replication checkpoint (*7*). At 20 min after the addition of sperm, we added various inhibitors of proteolysis and assayed for entrance into mitosis by a visual examination of nuclear morphology. Mitotic nuclei were distinguished from interphase nuclei by nuclear envelope breakdown (NEB) and by condensation of DNA to form mitotic chromosomes. To inhibit proteolysis, we used *N*-acetyl-Leu-Leu-norleucinal peptide (LLNL), which blocks the activity of the 26*S* proteasome, as well as methylated ubiquitin (me-Ub) and the ubiquitin mutant $Lys^{48} \rightarrow Arg^{48}$ (Ub-R48). Both of these ubiquitin derivatives inhibit the formation of poly-ubiquitin on target substrates and therefore block access to the proteasome. Figure 1A shows representative nuclei after 65 min in extracts treated with buffer, LLNL, recombinant wild-type ubiquitin, me-Ub, recombinant Ub-R48, or both me-Ub and wild-type ubiquitin. The inclusion of LLNL and the two altered versions of ubiquitin prevented NEB and therefore arrested the extract in interphase, whereas buffer and wild-type ubiquitin allowed entrance into mitosis (Fig. 1A). Methyl ubiquitin–mediated arrest was reversed upon coincubation with wild-type ubiquitin. To measure the extent of this arrest, we followed these reactions for up to 1 hour after the buffer-treated control had entered mitosis. LLNL produced a delay of 30 min, whereas the delays associated with methyl ubiquitin and Ub-R48 were 50 and 55 min, respectively (Fig. 1B). The addition of the proteolysis inhibitors at 20 min had no adverse affect on DNA replication (Fig. 1C). To determine when during interphase the degradation event occurs, we added Ub-R48 every 10 min to cycling extracts, starting at 20 min, and assayed for mitotic delay. The addition of Ub-R48 at 20 and 30 min produced a strong arrest, whereas the addition at 40 min or later had little, if any, effect (Fig. 1D). Therefore, sometime after the 40-min time point, the extracts were no longer dependent on proteolysis to enter mitosis. The measurement of DNA replication in these extracts showed that replication had completed at 45 min (*8*), indicating that the degradation event and the completion of DNA replication were temporally linked.

To examine a role for the SCF, we used a previously characterized dominant negative Cdc34 protein in which the conserved cysteine and leucine residues in the active site of the

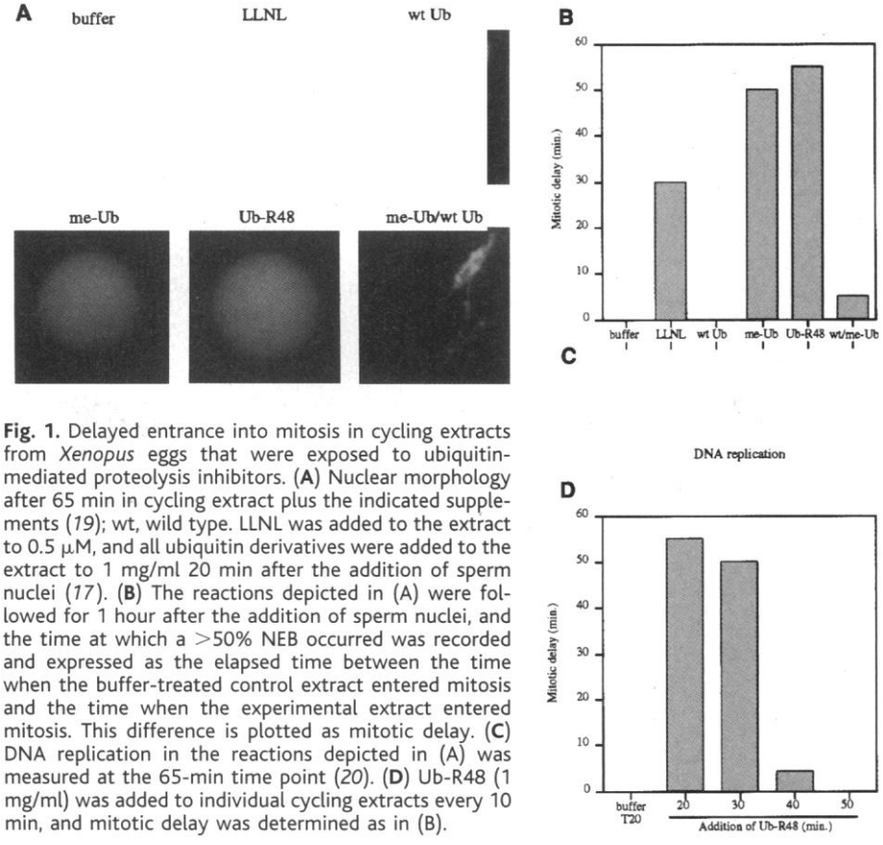

Fig. 1. Delayed entrance into mitosis in cycling extracts from *Xenopus* eggs that were exposed to ubiquitin-mediated proteolysis inhibitors. (**A**) Nuclear morphology after 65 min in cycling extract plus the indicated supplements (*19*); wt, wild type. LLNL was added to the extract to 0.5 μM, and all ubiquitin derivatives were added to the extract to 1 mg/ml 20 min after the addition of sperm nuclei (*17*). (**B**) The reactions depicted in (A) were followed for 1 hour after the addition of sperm nuclei, and the time at which a >50% NEB occurred was recorded and expressed as the elapsed time between the time when the buffer-treated control extract entered mitosis and the time when the experimental extract entered mitosis. This difference is plotted as mitotic delay. (**C**) DNA replication in the reactions depicted in (A) was measured at the 65-min time point (*20*). (**D**) Ub-R48 (1 mg/ml) was added to individual cycling extracts every 10 min, and mitotic delay was determined as in (B).

Table 1. Dominant negative Cdc34 protein delays entrance into mitosis. Recombinant proteins were added to cycling extracts at the indicated times and concentrations (*21*). The mitotic delay was determined as in Fig. 1B. Dash indicates that buffer, instead of recombinant protein, was added to the reaction; wt, wild type.

Protein	Concentration (μM)	Time of addition (min)	Mitotic delay (min)
Buffer	–	20	0
Cdc34 CL-S	20	20	50
Cdc34 wt	20	20	0
Cdc34 wt/CL-S	20 (each)	20	0
Cdc34 CL-S	5	20	5
Cdc34 Cl-S	15	20	15
Cdc34 CL-S	30	20	50
Cdc34 CL-S	20	40	5

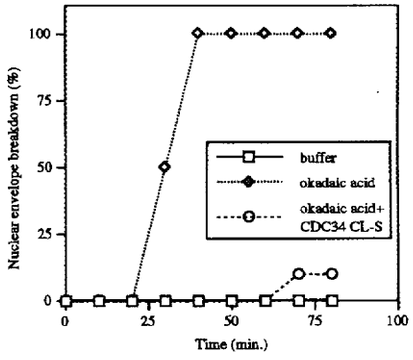

Fig. 2. Stabilization of the Cdc34 target during aphidicolin-induced arrest in S phase. Cycling extracts were supplemented with sperm nuclei and aphidicolin (100 μg/ml) and incubated for 60 min. The reaction was then divided into three parts and further supplemented with buffer, okadaic acid (200 nM), or okadaic acid and recombinant Cdc34 CL-S (*21*). The reactions were then monitored for NEB, and the percentage of NEB as a function of time after the addition of okadaic acid (or buffer) is plotted.

enzyme are mutated to serine (Cdc34 CL-S) (*5*). Recombinant protein was added to cycling extracts at 20 min, and G_2 arrest was assayed. Under these conditions, the addition of Cdc34 CL-S resulted in a G_2 arrest (50 min), whereas the wild-type Cdc34 protein had no effect relative to the buffer-treated control (Table 1). The effect of Cdc34 CL-S was reversed by the inclusion of an equimolar amount of wild-type protein (Table 1). We titrated Cdc34 CL-S into the reactions to determine the minimum concentration that would produce the G_2 arrest and found that 20 μM was the lowest concentration of Cdc34 CL-S that caused a maximal mitotic delay in the extracts (50 min) (Table 1). Like Ub-R48, the addition of Cdc34 CL-S at 40 min decreased the mitotic delay substantially (Table 1). Therefore, Cdc34-directed degradation of an inhibitor of mitosis is required for entrance into mitosis in *Xenopus*.

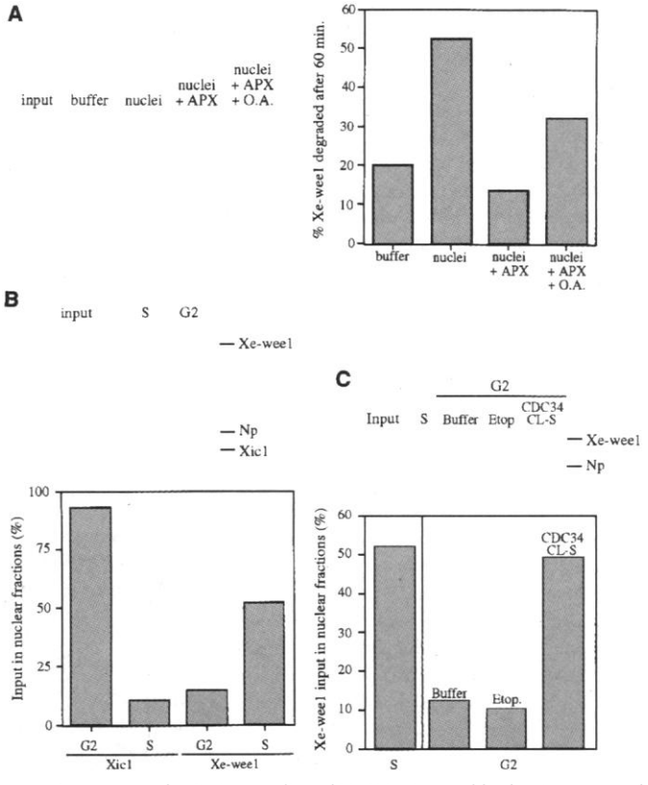

Fig. 3. Stabilization of *Xenopus* Wee1 in a replication checkpoint–dependent manner. **(A)** Xe-Wee1 degradation in cycling extracts. Xe-Wee1 that was labeled with ^{35}S was added to cycling extracts along with the indicated additional components (APX, aphidicolin at 100 μg/ml; nuclei, sperm nuclei at 2000 per microliter; O.A., okadaic acid at 200 nM). After 60 min, samples were removed from the extract and analyzed on protein gels to determine the amount of Xe-Wee1 degradation during the experiment. A representative experiment is depicted. The lane labeled "input" represents the amount of Xe-Wee1 that was present in the extract at the beginning of the experiment. Xe-Wee1 protein levels were quantified by phosphorimager analysis and plotted. The presence of okadaic acid resulted in an upward shift in mobility of Xe-Wee1, possibly due to increased phosphorylation. **(B)** Representative degradation assay showing the amount of substrate remaining in the nuclei-containing sediment fractions after a 60-min incubation in the indicated extract (S, S phase–arrested extract; G_2, G_2 extract). The lane labeled "input" represents 15% of the starting material. Nucleoplasmin (Np), a stable protein, was used to normalize the amounts of Xe-Wee1 and Xic1 in the sedimented fractions (*22*). The amount of Xic1 and Xe-Wee1 remaining after a 60-min incubation in the given extract was quantified by phosphorimager analysis and plotted. **(C)** Representative degradation assay showing the amount of substrate remaining in the nuclei-containing sediment fractions after a 60-min incubation in the indicated extract. Assays were performed in S phase extract, G_2 extract supplemented with buffer or etoposide (Etop.) (20 min after the addition of sperm nuclei), or G_2 extract containing recombinant Cdc34 CL-S (added at 60 min, along with the ^{35}S-labeled proteins).

MPF activation is the rate-limiting event for entrance into mitosis (*10*). During interphase, the protein kinases Wee1 and Myt1 phosphorylate Thr^{14} and Tyr^{15} of the Cdc2 subunit of MPF, which inactivates MPF kinase activity (*11*, *12*). The Cdc25 phosphatase reverses these modifications and activates MPF (*13*). Therefore, it is the balance between the activities of the inhibitors Wee1 and Myt1 (*11*, *12*) and the activator Cdc25 (*13*) that ultimately controls MPF activation

The phosphatase inhibitor okadaic acid has been shown to induce mitosis in the presence of the DNA replication checkpoint (*9*), thereby overriding the control mechanisms that normally prevent the activation of MPF during S phase. Under normal cycling conditions, the extracts lose Cdc34 CL-S sensitivity after 40 min, by which time DNA replication is nearly complete. If extracts were incubated with aphidicolin [which blocks DNA replication and induces checkpoint control (*7*)] for 60 min and then treated with okadaic acid, they entered mitosis ~20 min later (Fig. 2). However, if Cdc34 CL-S was added with okadaic acid at the 60-min time point, then premature mitosis did not occur (Fig. 2). This result demonstrates that okadaic acid cannot prevent the Cdc34 CL-S–induced G_2 arrest. More important, it also shows that the Cdc34 target, which is normally degraded by the time that S phase is complete (45 min), was still present at the 60-min time point when DNA replication was inhibited. Thus, degradation of the Cdc34 target appears to be linked to the completion of S phase.

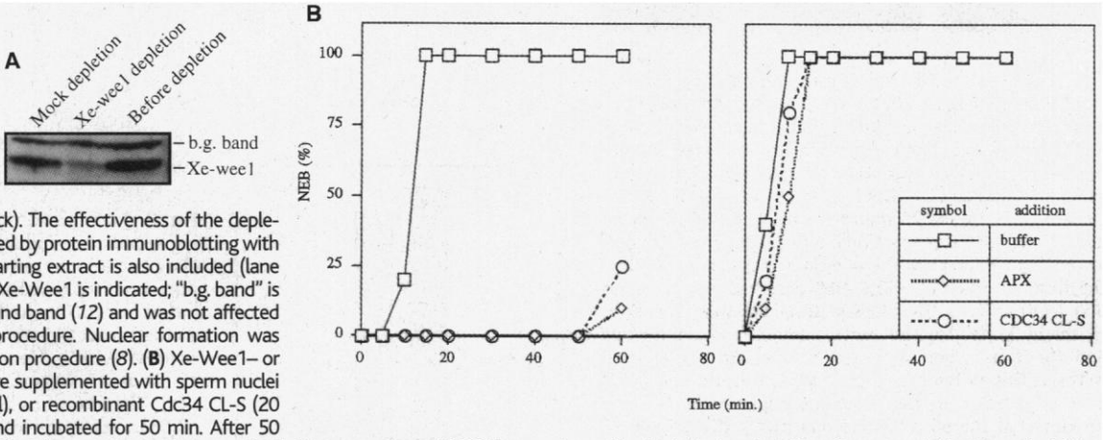

Fig. 4. Loss of Cdc34 CL-S sensitivity and replication checkpoint control in extracts depleted of Xe-Wee1. **(A)** Interphase extracts were depleted (*23*) with antibodies to Wee1 or with nonspecific control antibodies (Mock). The effectiveness of the depletion procedure was monitored by protein immunoblotting with antibodies to Wee1. The starting extract is also included (lane labeled "Before depletion"). Xe-Wee1 is indicated; "b.g. band" is a previously noted background band (*12*) and was not affected by the immunodepletion procedure. Nuclear formation was not disturbed by the depletion procedure (*8*). **(B)** Xe-Wee1– or mock-depleted extracts were supplemented with sperm nuclei and buffer, APX (100 μg/ml), or recombinant Cdc34 CL-S (20 μM added 20 min later) and incubated for 50 min. After 50 min, recombinant cyclin B (30 nM) was added to induce mitosis. The percentage of NEB from each reaction is plotted against time; 0 represents the time of addition of cyclin B.

and entrance into mitosis (*10*). We have defined an activity in cycling extracts that must be degraded to allow mitosis, and we have considered that Wee1 might represent this activity. *Xenopus* Wee1 (Xe-Wee1) (*12*) was labeled with ^{35}S methionine and added to cycling extracts containing buffer, sperm nuclei, sperm nuclei plus aphidicolin, or sperm nuclei plus aphidicolin and okadaic acid. After 60 min, the amount of Xe-Wee1 that had degraded during the incubation period was determined. In the absence of sperm nuclei, 20% of the input Xe-Wee1 had been degraded, whereas 52% was degraded when nuclei were added (Fig. 3A). If DNA replication was prevented by the inclusion of aphidicolin, then only 13% of the input was degraded, and okadaic acid increased degradation to 34% in aphidicolin-treated reactions (Fig. 3A). This indicates that Xe-Wee1 was substantially degraded only when nuclei were present, that inhibiting DNA replication affects Xe-Wee1 stability, and that okadaic acid overrides this effect and promotes Xe-Wee1 degradation.

Xe-Wee1, like Wee1 kinases from other organisms, is a nuclear protein (*12*). Because the addition of nuclei to the extract facilitated Xe-Wee1 degradation, we reasoned that the nuclear pool of Xe-Wee1 may be particularly susceptible to proteolysis. We therefore measured the stability of Xe-Wee1 within nuclei formed in S phase–arrested extracts (S extracts) in relation to nuclei from extracts that had completed S phase (G_2 extracts). Nuclei were added to extracts containing cyclohexamide (to block entry into mitosis) and were supplemented with either buffer or aphidicolin. After 60 min, we added ^{35}S-labeled proteins and incubated for a further 60 min, at which time nuclei were isolated from the extract by centrifugation through a sucrose cushion. Protein levels from isolated nuclei were then determined. We compared the stability of Xe-Wee1 to that of Xic1, a protein previously shown to be degraded in a nuclei- and a Cdc34-dependent manner (*5*). Xic1 was rapidly degraded in S phase nuclei and was stable in G_2 nuclei (Fig. 3B). This indicates that Xic1 degradation stops after the completion of DNA replication. In contrast, Xe-Wee1 was moderately unstable in S phase nuclei (52% of input remained after 60 min) but is almost completely degraded in G_2 nuclei (12% of input remained after 60 min) (Fig. 3B). These results show that the stability of Xe-Wee1 within nuclei was increased substantially by the presence of unreplicated DNA and suggest that the DNA replication checkpoint may attenuate Xe-Wee1 degradation. To see if this is specific to the replication checkpoint, we induced the DNA damage checkpoint in extracts and analyzed Xe-Wee1 stability under these conditions. To induce the damage checkpoint, we used etoposide, a known DNA damage–inducing agent (*14*). The addition of etoposide to cycling extracts elicited a strong delay on entrance into mitosis but did not effect DNA replication (*8*). Xe-Wee1 stability in etoposide-treated nuclei was nearly identical to that in the untreated G_2 nuclei (Fig. 3C). Xe-Wee1 stabilization in replication checkpoint–arrested extracts was therefore specific for the replication block and was not a general consequence of cell cycle arrest. If Xe-Wee1 is the Cdc34 target for entrance into mitosis, then Cdc34 CL-S should increase the stability of Xe-Wee1 in G_2 nuclei. Indeed, the addition of Cdc34 CL-S stabilized Xe-Wee1 to the amounts seen in S phase nuclei (Fig. 3C). We conclude that Cdc34-directed proteolysis of Xe-Wee1 may be regulated to occur only after the completion of S phase.

If Xe-Wee1 is the Cdc34 target that must be degraded to allow MPF activation, then the removal of Xe-Wee1 should render the system insensitive to the inhibitory effects of Cdc34 CL-S. We depleted Xe-Wee1 from extracts and then assayed the effect of Cdc34 CL-S on entrance into mitosis. Depleted extracts entered mitosis at the same time, regardless of the presence of Cdc34 CL-S, whereas mock-depleted control extracts underwent a Cdc34 CL-S–dependent mitotic delay (Fig. 4). We also assayed for induction of the replication checkpoint control in extracts depleted of Xe-Wee1 and found that the replication checkpoint could not delay mitosis in the absence of Xe-Wee1.

Our findings present a framework for understanding how mitosis is dependent on the completion of S phase. We propose that, during DNA replication, a signal may be generated [possibly through checkpoint kinases such as Cds1 or Chk1 (*15*, *16*)] that prevents the Cdc34-mediated degradation of Wee1. After replication, this signal attenuates, and Wee1 becomes a Cdc34 degradation substrate. This shift in the balance toward Cdc25 triggers MPF activation, and mitosis ensues. This model for replication checkpoint control is consistent with findings from a study of *Schizosaccharomyces pombe*, in which the Wee1-related kinase Mik1 is stabilized during S phase arrest in a checkpoint-dependent manner (*15*).

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20. DNA replication assays were performed as described in (*7*).
21. Recombinant wild-type and Cdc34 CL-S proteins were prepared as described in (*5*).
22. To assay protein degradation in nuclei, we mixed freshly prepared interphase extracts [cycling extracts and cyclohexamide (100 μg/ml)] with sperm nuclei (2000/μl) and incubated either with (S extract) or without (G_2 extract) aphidicolin for 1 hour. In vitro translated (IVT) proteins (2 μl) that were labeled with ^{35}S were added to 25 μl of extract, and incubations were continued for 60 min. The extracts were diluted with 500 μl of nuclear isolation buffer (NIBS) (*17*), loaded onto a cushion of 200 μl of NIBS plus 0.5 M sucrose, and centrifuged for 5 min at 6000*g*. The supernatant was removed, and the sedimented fractions were resuspended in SDS–polyacrylamide gel electrophoresis (PAGE) sample buffer. After SDS-PAGE, the gels were fixed and dried, the amount of radioactivity present in each protein band was quantified with a phosphorimager, and the data were analyzed with the NIH ImageQuant software package. To determine the percent degradation for each sample, we normalized the amount of material in the sedimented fractions by the inclusion of IVT nucleoplasmin, which we assumed to be a stable protein in this assay. The actual values for Wee1 and Xic1 in the pellet fractions were then expressed as a fraction of this value to get the percentage of input in nuclear fractions. In vitro translation of plasmids encoding Xic1 (*18*), Xe-Wee1 (*12*), and nucleoplasmin (*8*) was done with a coupled transcription/translation kit (Promega, Madison, WI) according to the manufacturer's instructions.
23. Interphase extracts were depleted with antibodies 725 and 1532 to Xe-Wee1 (*12*) or nonspecific control immunoglobulin G (Sigma). For each depletion, 100 μl of extract was applied to 50 μl of packed protein A–Sepharose coupled to antibody and incubated at 4°C with rotation for 1 hour. The beads were then gently centrifuged, and the extract was removed. Extracts were supplemented with the indicated reaction components and incubated for 50 min before the addition of 30 nM recombinant human cyclin B.

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