other organisms are semidominant (*1*), as is *toc1*. The distribution of periods in the large F_2 population of the mapping cross (Fig. 1D) suggests a 1:2:1 segregation with mean periods of 20.7, 23.0, and 24.2 hours, corresponding to *toc1*/*toc1* homozygote, putative *toc1*/*TOC1* heterozygote, and *TOC1*/*TOC1* homozygote classes, respectively.

Clock mutations in other species commonly affect several rhythmic markers in parallel (*1*), suggesting that a single oscillator can control many outputs. *Arabidopsis* exhibits several circadian markers, including rhythms in stomatal aperture (*16*), floral induction (*17*), the expression of nuclear genes (*18*), and the movements of cotyledons and primary leaves (*19*). We used an automated video imaging system to monitor leaf movements in *toc1* plants and the transgenic parent line (*20*). The rhythm of leaf position for typical wild-type and *toc1* leaves is shown in Fig. 3. The leaf movement rhythm had a slightly longer period in the wild type than the rhythm of *cab2::Ω::Luc* luminescence (25.2 ± 1.0 hours, $n = 24$, compared with 24.7 hours for *CAB*). The period of the leaf movement rhythm was significantly shorter in *toc1* plants (23.3 ± 1.3 hours, $n = 18$), indicating that *toc1* encodes a component common to both the *CAB* and leaf movement rhythms.

Mutants in the *det* class are likely to show photomorphogenetic phenotypes and elevated *CAB* expression in dark-grown plants, in addition to their short periods in LL (*8*, *10*). Neither phenotype is found in *toc1* plants. Thus, if *TOC1* is part of the input pathway or pathways defined by *det* and phytochrome mutations, its function must follow a branch point that separates signals to the clock from signals to morphogenesis and to the regulators of *CAB* expression level. Alternatively, *toc1* may affect another input pathway or directly affect the circadian oscillator.

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4. Seed homozygous for the *cab2::Ω::Luc* construct were soaked in 0.4% ethylmethane sulfonate for 12 hours at room temperature and sown in soil (M1 seed). Approximately 4500 M1 plants flowered. Self-fertilized seed (M2 seed) were harvested from pools of approximately 60 M1 plants.
5. The luminescence from 200 M2 seedlings from each of 39 M1 pools (total of ~8000 M2 seedlings) was assayed by video imaging. Images were recorded when luminescence began to rise (19.5 hours after transfer to LL), close to the peak of luminescence (27.5 hours), and again near the trough (37 hours), when luminescence in control seedlings had returned to the level at 19.5 hours. Seedlings were returned to LL after each image.
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21. We thank K. W. Smith and D. Gerber for technical assistance, M. Straume and T. M. Breeden for programming, and D. Somers for critical reading of the manuscript. Supported by grants from the NSF Center for Biological Timing to S.A.K. and NIH grant GM44640 to N.-H.C. A.J.M. was supported by a William O. Baker Fellowship, through a grant from the Mellon Foundation. S.A.K. is supported by an award from the W. M. Keck Foundation.

8 September 1994; accepted 6 December 1994

The Regulation of Circadian Period by Phototransduction Pathways in *Arabidopsis*

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Transgenic *Arabidopsis* plants expressing a luciferase gene fused to a circadian-regulated promoter exhibited robust rhythms in bioluminescence. The cyclic luminescence has a 24.7-hour period in white light but 30- to 36-hour periods under constant darkness. Either red or blue light shortened the period of the wild type to 25 hours. A phytochrome-deficient mutation lengthened the period in continuous red light but had little effect in continuous blue light, whereas seedlings carrying mutations that activate light-dependent pathways in darkness maintained shorter periods in constant darkness. These results suggest that both phytochrome- and blue light–responsive photoreceptor pathways control the period of the circadian clock.

The fluctuations of light quality and fluence rate in the natural day-night cycle are particularly important to plants, which depend on light as a source of energy, as a developmental signal, and as a cue for tropic movements. Multiple photoreceptors allow the plant to sense the light environment; these include the phytochromes (red–far red receptors), blue light receptors, and at least one ultraviolet-B receptor (*1*). Photoreceptor pathways and circadian systems interact at several levels in higher plants (*2*), though the details of these interactions vary widely among species. The circadian clock has been reported to modulate some photoreceptor functions (*3*). Light pulses or steps control the phase of the clock, mediating entrainment to the day-night cycle (*4*), whereas continuous photoreceptor ac-

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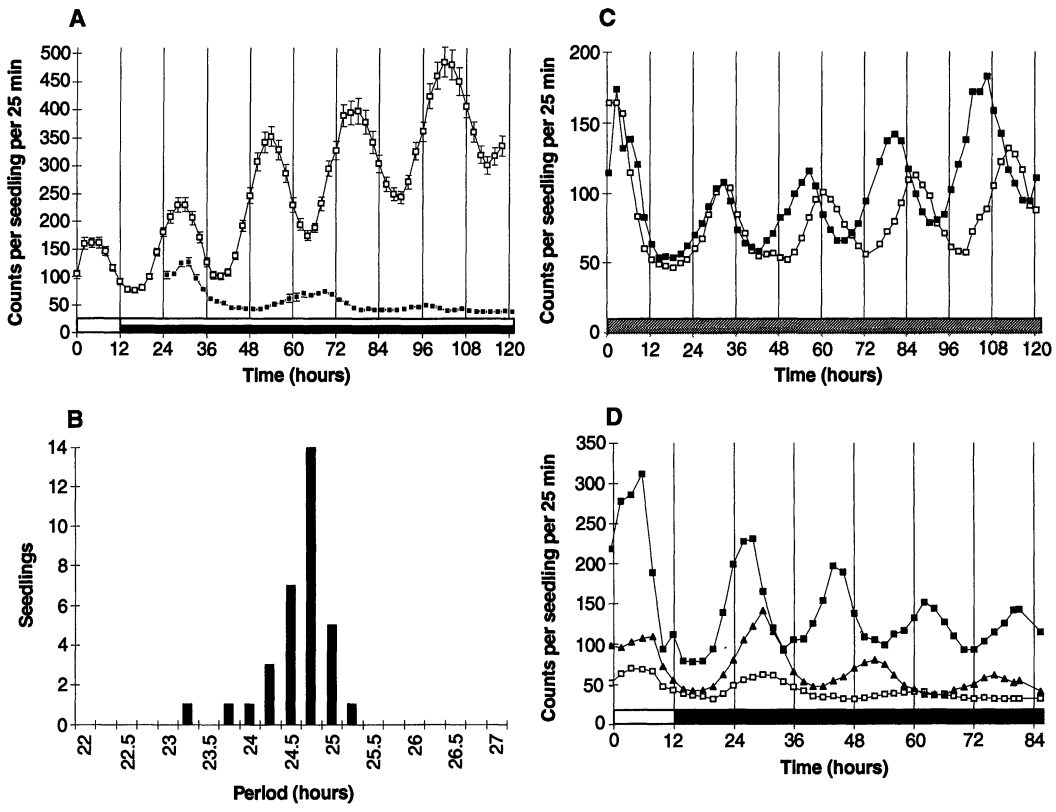

Fig. 1. Circadian regulation in *cab2*::Ω::*Luc* seedlings. (**A**) Luminescence of *cab2*::Ω::*Luc* seedlings in LL and DD. Populations of 33 seedlings were imaged in LD and LL (open symbols) or DD (filled symbols). Arithmetic means were calculated from single-seedling records; error bars represent one SEM. The trace in LL shows a particularly strong increase in luminescence over the timecourse. Error bars for DD are often within the symbols. These (and subsequent) data have not been corrected for background counts (for an image, typically 35 counts per seedling). The open box on the time axis indicates white light, the filled box darkness. (**B**) Period distribution in LL. Periods were estimated (*11*) from the LL data in (A). Period bins are labeled with the upper bound. (**C**) Luminescence of *hy1* mutant in R. Seedlings homozygous for *hy1* (F_3 generation, open symbols) and parental *cab2*::Ω::*Luc* seedlings (filled symbols) were grown under LD and assayed after transfer to R. Arithmetic means were calculated from luminescence data for 28 individual seedlings. Hatched box, red light. (**D**) Luminescence of *det1*, *det2*, and *cop1-6* mutants in DD. Seedlings homozygous for *det1* (F_3 generation, filled squares), *det2* (F_2 generation, open squares), or *cop1-6* (F_2 generation, filled triangles) were transferred from LD to DD and imaged at the times indicated. Arithmetic means were calculated from luminescence data for 28 to 31 individual seedlings. Open box, white light; filled box, darkness.

tivation modulates the period (*5*). Downstream targets of regulation often exhibit rhythmic responsiveness to light signals, for example, in the photoperiodic control of flowering (*6*) or in the expression of the chlorophyll *a/b*–binding protein (CAB) genes (*7*). The effect of light on the amplitude of cycling is often manifest as "damping," the collapse of amplitude to the point of arrhythmicity or inactivity (*1*, *2*).

Fusions between a fragment of the *Arabidopsis* *CAB2* promoter and the firefly luciferase (*Luc*) coding region provide a bio- luminescent reporter of CAB transcriptional activity in individual, intact plants (*8*). We have used this marker in transgenic *Arabidopsis* (*9*) to define some of the interactions between phototransduction pathways and the circadian oscillator. Transgenic *cab2*::Ω::*Luc* seedlings were grown under 12-hour light-dark (LD) cycles, and then transferred to continuous light (LL) or darkness (DD) (*10*). Luminescence was assayed by video imaging (Fig. 1A) (*9*). The peak of luciferase-generated luminescence occurred 3 to 6 hours after lights on, and the amplitude of cycling (peak to trough) in LD is 6- to 10-fold after background subtraction. Seedlings in LL had a mean period of 24.7 hours (SD 0.4 hour; Fig. 1B) (*11*). The cycles of plants transferred to DD, in contrast, exhibited rapid damping and a long-

Table 1. Periods of wild-type and mutant seedlings under DD, B, R, and LL. Seedlings were transferred to the conditions indicated in each section and assayed by video imaging. Periods were estimated for each seedling (except where indicated). Variance-weighted period means (<per>) and standard deviations were calculated for each population (*11*). Standard errors of the mean (<SE>) are presented to correct for variations in the number of seedlings in each population (*n*). The generation of crossed progeny used in each experiment is indicated. Replicated data are derived from independent experiments.

Seedling	DD			B			R			LL		
	<per>	<SE>	*n*	<per>	<SE>	*n*	<per>	<SE>	*n*	<per>	<SE>	*n*
					cab2::Ω::Luc *transgenic parent*							
cab2::Ω::*Luc*	30*	1.72	33	25.0	0.07	32	25.1	0.05	33	24.7	0.07	33
cab2::Ω::*Luc*	36*	1.48	28	25.1	0.04	33	25.0	0.06	33	24.6	0.06	33
					Columbia control, hy1, *and* det1							
Columbia F2	31*	0.34	35	24.6	0.07	26	25.3	0.09	22	24.4	0.07	25
hy1 F3-7	43*	11.87	19	24.2	0.11	30	27.6	0.18	28	24.8	0.14	30
hy1 F3-4	30*	1.77	24	24.2	0.15	24	25.8	0.19	25	24.4	0.13	25
det1 F4-2	18.8	0.17	27	19.5	0.07	23	20.5	0.21	26	20.9	0.25	22
det1 Tx2 F2-1†	18.2	0.16	18	20.2	0.15	24	19.2	0.16	21	20.4	0.07	26

*Period estimation is inaccurate for the wild type in DD, as suggested by the high SE values, due to the rapid damping of luminescence. †The *det1* plants that carried the transgene in the F_2 of the first cross were back-crossed again to *det1*. The F_2 of the second cross (Tx2 F2) is 100% mutant and segregates for the transgene.

Table 2. Periods of mutant seedlings under R and DD. Abbreviations, procedures, and calculations are as described in Table 1. The dash indicates that the calculation is not applicable.

Seedling	<per>	<SE>	*n*
	hy1 *under R*		
hy1 F2*	26.6	0.21	15
hy1 F3	26.5	0.26	7†
	det1, det2, *and* cop1-6 *under DD*		
det1 F1	31‡	–	1†
det1 F2*	18.5	0.24	10
det1 F3-1	18.4	0.06	31
det1 F3-1	18.0	0.12	11
det1 F3-8	17.8	0.31	11
det1 Tx2 F2-1§	17.8	0.15	10
det1 Tx2 F2-2§	17.8	0.20	6
det1 Tx2 F2-3§	17.4	0.14	8
det2 F2*	29.3	0.67	23
det2 F3	28.9	1.62	14
cop1-6 F2*	23.8	0.27	22
cop1-6 F3	22.6	0.20	12

*Homozygous mutants were selected on the basis of morphological phenotypes. †Period estimates derived from total luminescence of 25 to 33 seedlings from each family; *n* is the number of families, not the number of individuals. ‡Period estimation is inaccurate for the wild type in DD, as suggested by the high SE values, due to the rapid damping of luminescence. §The *det1* plants that carried the transgene in the F_2 of the first cross were back-crossed again to *det1*. The F_2 of the second cross (Tx2 F2) is 100% mutant and segregates for the transgene.

er period (Fig. 1A). The first peak of luminescence in DD was at the expected phase from the preceding LD cycle and reached 60 to 70% of the maximal LD luminescence level. The second peak of luminescence was broader, was centered at 66 hours, and was reduced to 20 to 30% of the LD maximum. The third peak occurred around 96 hours. Thus, the period in DD ranged from 30 to 36 hours. The rapid damping of *CAB* expression level in DD is consistent with our previous analysis of *cab2::Luc* in tobacco (8) and is probably due to the decline in photoreceptor activation (7).

Factors that may lengthen the period in DD relative to LL include the coupling of the *cab2* promoter to different circadian oscillators in the light as compared with darkness. Alternatively, light may modulate the period of a single oscillator, in which case conditions that reduce the activity of phototransduction pathways will lengthen the period, and conditions that increase their activity will shorten the period. We used lighting conditions and photomorphogenetic mutants to control the activity of phototransduction pathways. White light fluence rates up to 600 μmol m^{-2} s^{-1} did not cause any decrease in the period of wild-type plants (8) (our standard conditions were 50 to 60 μmol m^{-2} s^{-1}). The phototransduction pathways to the circadian oscillator may therefore be saturated under standard conditions. The *cab2::Ω::Luc* seedlings were assayed after transfer from LD (*10*) to constant red (R) or blue (B) light (Table 1) (*12*). Both B and R conditions shortened the period to almost the same extent as LL, and the other parameters of the rhythm were also very similar in R, B, and LL (*13*).

We next analyzed the involvement of the photomorphogenetic loci *HY1*, *DET1*, *DET2*, and *COP1* in period control. The *hy1-100* (long hypocotyl) mutant lacks spectrophotometrically detectable phytochrome in etiolated seedlings (*14*) and probably contains very low levels of all phytochromes. The *det* (de-etiolated) and *cop* (constitutively photomorphogenetic) seedlings exhibit characteristics of light-grown plants even when grown in darkness, including the expression of *CAB* genes (*15–17*). We also crossed the *cab2::Ω::Luc* transgene into the wild-type parent of these mutants (ecotype Columbia) (*18*). Seedlings were grown in standard conditions (*10*) and assayed after transfer to DD, R, B, or LL (Table 1). Periods and other parameters in the Columbia ecotype were very similar to the parental transgenic line under all conditions (*13*).

The *hy1* plants had a period similar to that of the wild type under LL, indicating that only very small amounts of phytochrome, if any, are required for white light to affect the period. The period of *hy1* under R, in contrast, was significantly lengthened (Tables 1 and 2). The *hy1-100* mutation is probably leaky, and the low amount of phytochrome present may be sufficient to cause a shorter period under R (26.5 hours) relative to the wild type in DD (30 to 36 hours). It is unlikely that the blue light pathway is activated under our R conditions (*12*). These results indicate that one or more phytochromes modulate the period in wild-type plants under R. The *hy1* seedlings did not have long periods in B, indicating that other, blue-responsive photoreceptors are involved in period control. The small decrease in the period in *hy1* relative to the Columbia control under B ($P < 1\%$ and $P < 5\%$, Table 1) suggests that phytochromes may, if anything, slightly lengthen the period in these conditions. Maximum luminescence for *hy1* and parental transgenic seedlings was reduced 40% 30 hours after transfer to R or B (*13*), relative to LL (Fig. 1, A and C), suggesting that both blue-responsive and red-responsive photoreceptors affect *CAB* gene expression level in LL. Luminescence levels in *hy1* under DD, B, and LL were very similar to that of the transgenic parent (*13*), though the upward trend in luminescence under R was less than that of the parent line (Fig. 1C).

The period of *det1* mutants was very short in DD (about 18 hours) and slightly longer (19 to 20 hours) in B, R, and LL (Tables 1 and 2). This phenotype is strictly recessive, like the morphological phenotypes of *det1* (*15*). The *det2* and *cop1-6* mutations also shortened the period in DD, consistent with their proposed role in activating phototransduction pathways independently of light (*19*). The very short period of *det1* contrasts with the period of wild-type plants grown in high-fluence white light (about 24.7 hours) (8), suggesting that the *det1* lesion bypasses a saturating point in the phototransduction pathway or eliminates a period-lengthening factor. Another possibility is that the *DET* and *COP* genes encode pacemaker components, in which case the null mutations will exhibit arrhythmic *CAB* expression.

The *det1*, *det2*, and *cop1-6* mutants all entrained to LD (Fig. 1D), indicating that the circadian system retains some light sensitivity, although in *det1* the amplitude of cycling was reduced. After transfer to DD, the peak luminescence decreased by about 50% in the first day and stabilized thereafter (Fig. 1D). Both *det2* and *cop1-6* also affected the period (Table 2) and damping (Fig. 1D) in DD. The rank order in period (*det1*, 18 hours; *cop1-6*, 23 to 24 hours; *det2*, 29 hours; parent, 30 to 36 hours) and in the rate of damping in DD is the same as the ranking with respect to photomorphogenetic phenotypes in dark-grown plants for these alleles (*15–17*). The coordinate effects on these traits suggest that they are due to a single, aberrant function in each mutant. We cannot assess the relative importance of the mutated genes for the control of the period, as the alleles we used are variously weak (*cop1-6*) (*17*), intermediate (*det1-1*) (*15*), and of unknown strength (*det2-1*) (*16*).

CAB expression induced by red light pulses in etiolated tissue shows periods greater than 24 hours in some species (*7, 8*). As the activity of phototransduction pathways decreases after the end of the light pulse, period lengthening may be caused by a similar mechanism to that operating in green tissue transferred to DD. Thus, *CAB* expression can cycle with a range of periods. Both phytochrome and nonphytochrome blue-responsive photoreceptors are involved in maintaining periods close to 25 hours under red and blue light. Mutations that influence phototransduction pathways affect the period in a manner consistent with their effects on phototransduction, as inferred from their biochemical defects and photomorphogenetic phenotypes. These results suggest that the 30- to 36-hour periods of the wild type in DD reflect a period lengthening of the same oscillator that is active in LL, mediated by the loss of activation in several phototransduction pathways.

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10. Seeds for in vivo imaging were sown in a grid pattern and stored for 4 to 7 days in dim light at 4°C. Seedlings were grown for 5 days in a controlled environment chamber at 22°C, under 50 to 60 μmol m^{-2} s^{-1} fluorescent light in LD. At dawn on the sixth day of growth, the seedlings were transferred to constant conditions and imaged as described (*9*).
11. For estimating the period, we used a modified sine wave function of the form

$$L(t) = (c_0 + c_1 t) + (amp_0 + amp_1 t)\sin(2\pi t/T - \phi)$$

where L is luminescence, t is time, T is the period estimate, and ϕ is the estimate of the phase at $t = 0$. The variable c_0 is the estimated value of the luminescence level at $t = 0$, and c_1 is an estimate of the linear rate of change in luminescence level; amp_0 is the estimated value of the cycling amplitude at $t = 0$, and amp_1 is an estimate of the linear rate of change in cycling amplitude. The fitting is based on nonlinear least squares minimization of the variance of fit. Variance-weighted means of period estimates (⟨T⟩, or <per> in Tables 1 and 2) within a group of seedlings were calculated according to Bevington [P. R. Bevington, *Data Reduction and Error Analysis for the Physical Sciences* (McGraw-Hill, New York, 1969), p. 73] and variance-weighted standard deviations by a similar method. Full details of these programs are available from the authors. Significance levels were taken as 5%.
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20. We thank K. W. Smith for assistance, D. Gerber for imaging, D. Somers for critically reading the manuscript, and T. M. Breeden for programming. Supported by grants from the NSF Center for Biological Timing to S.A.K. and NIH grant GM44640 to N.-H.C. A.J.M. was supported by a William O. Baker Fellowship, through a grant from the Mellon Foundation. S.A.K. is supported by an award from the W. M. Keck Foundation.

8 September 1994; accepted 6 December 1994

A Role for Exonuclease I from *S. pombe* in Mutation Avoidance and Mismatch Correction

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Exonuclease I (Exo I) from *Schizosaccharomyces pombe*, a 5′ → 3′ double-stranded DNA exonuclease, is induced during meiotic prophase I. The *exo1* gene is a member of a family of related DNA repair genes, including *RAD2/rad13/xpgc* and *YKL510/rad2*, conserved from yeast to humans. An *exo1* mutant displays a mutator phenotype and alters activity of the *ade6-M387* marker effect. These results suggest that Exo I acts in a pathway that corrects mismatched base pairs.

Exonucleases act in multiple pathways of DNA repair and recombination. Enzymes removing only one strand of duplex DNA are postulated to create single-stranded DNA (ssDNA) that, in turn, can act as a substrate for homologous pairing and strand-exchange activities during recombination and recombinational repair of broken chromosomes (*1*, *2*). *Escherichia coli* and its phages encode such strand-specific exonucleases; strains lacking these enzymes are deficient in various aspects of recombination and repair (*1*). In eukaryotes, identified intermediates in double-strand break repair are consistent with the action of 5′ → 3′ double-stranded DNA (dsDNA) exonucleases (*3*).

Exonucleases are also implicated in the late steps of homologous recombination, namely the correction of mismatched base pairs resulting from hybrid DNA formation between single stands of chromosomes carrying different point mutations. Mismatch correction influences the outcome of recombination in two-factor intragenic crosses. Close genetic markers are often covered by a single tract of heteroduplex DNA and can form recombinants only if the two resulting mismatches are corrected independently. In addition, gradients of gene conversion frequencies across the *ARG4* and *HIS4* genes of *Saccharomyces cerevisiae* are at least in part a result of mismatch correction (*4*, *5*). In *E. coli*, the correction of mismatches caused by replication errors involves the combined action of helicases and ssDNA exonucleases (*6*), but no eukaryotic exonucleases acting in these processes have been identified yet.

We have previously described the activity of a meiotically induced 5′ → 3′ dsDNA exonuclease, Exo I, from *S. pombe* (*7*). Exo I acts on both double-strand ends and nicks and is a good candidate for an activity required in the processes discussed above. Here, we present evidence that Exo I contributes to mismatch correction.

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The *exo1* gene was cloned by polymerase chain reaction with degenerate primers that were derived from a peptide sequence of the Exo I protein (*8*). From the 2-kb insert of one of the *exo1* clones, pXON4, the DNA sequence of both strands was determined (GenBank accession number L35174). A single, uninterrupted open reading frame (ORF) encoding a putative polypeptide of 63,866 daltons was identified (*9*). The size of purified Exo I was estimated by SDS–polyacrylamide gel electrophoresis to be approximately 36,000 daltons (*7*). We presume that previously purified Exo I is a proteolytic product.

By comparison of the predicted Exo I amino acid sequence to those in databases, we found it was homologous to that of previously reported proteins (Fig. 1). The *S. cerevisiae RAD2* gene, which encodes a ssDNA endonuclease, and its *S. pombe* and human homologs *rad13* and *xpgc*, respectively, all function in ultraviolet (UV) excision repair (*10*–*12*). Although these genes are assumed to encode functional homologs (*13*), the strong sequence homologies are limited to discrete blocks interspersed with regions of poor conservation (*14*). The *exo1* gene is a composite of some of these blocks but with less sequence conservation than between *RAD2*, *rad13*, and *xpgc*. Another *S. pombe* gene involved in UV excision repair, *S.p. rad2* (*15*, *16*), and its presumed homologs from *S. cerevisiae*, *YKL510* (*17*), and from humans, *H.s. rad2* (*16*), have the same composite structure as *exo1* but are much more closely related to each other than to *exo1* (Fig. 1). The *exo1* gene appears to represent a new, third class in this family of related genes.

To explore the basis of the rapid rise and decrease of enzyme activity during meiosis (*7*), we carried out a Northern (RNA) analysis of RNA isolated from cells at different points during meiosis (Fig. 2A). At 4, 5, and 6 hours after induction of meiosis, the amount of *exo1* transcript increased approximately 10-fold, peaking at 5 hours. This increase roughly parallels the changes detected in dsDNA exonuclease activity detected in cell extracts (*7*) and suggests con-