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Identification and Expression of *uvi31*$^+$, a UV-Inducible Gene From *Schizosaccharomyces pombe*

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The *Schizosaccharomyces pombe uvi31*$^+$ gene has been previously isolated as a UV-inducible gene [Lee JK et al. (1994) Biochem Biophys Res Commun 202:1113–1119]. This gene encodes a protein of about 12 kDa with 57% amino acid sequence similarity to *Escherichia coli* BolA protein which is known to be involved in switching between the cell elongation and septation systems during the cell division cycle. The putative *Mlu*I cell cycle box (MCB), SWI4/6-dependent cell cycle box (SCB), and gearbox elements are found in the upstream region of *uvi31*$^+$ gene, suggesting that this gene shows the cell cycle–regulated and growth phase–dependent expression. Interestingly, the level of *uvi31*$^+$ transcript varies throughout the cell cycle, peaking in G1 phase before septation, and also shows the growth phase–dependent pattern during cellular growth, increasing maximally at the diauxic shift phase just before stationary phase. Furthermore, the transcript level of this gene is raised after S phase arrest, and is also increased maximally at 4 hr after UV irradiation of 240 J/m^2. These results suggest that the delayed induction of *uvi31*$^+$ gene after UV irradiation may be caused by cell cycle control of this gene after DNA replication checkpoint arrest. Thus, the *uvi31*$^+$ gene may play a role in controlling the progress of the cell cycle after DNA damage (UV irradiation). Environ. Mol. Mutagen. 30:72–81, 1997

Key words: *Schizosaccharomyces pombe; uvi31*$^+$; DNA damage; UV-inducible; periodic expression; cell cycle control

INTRODUCTION

Many genes that are transcriptionally activated in response to DNA damage have been identified, including several involved in DNA repair, cell cycle arrest, and DNA replication [Zhou and Elledge, 1993; Friedberg et al., 1995], and it has been suggested that there is more than one common DNA damage response pathway to regulate these genes. These DNA damage response pathways are essential to maintain genomic integrity and to duplicate genetic material with the highest fidelity [Carr and Hoekstra, 1995]. This is well understood in the SOS response of *Escherichia coli* [Friedberg et al., 1995].

Cells finish DNA replication, DNA repair, and chromosome segregation before cell division using cell cycle controls which can detect the failure to complete replication, repair, or spindle assembly to arrest the progress of the cell cycle at one of three checkpoints [Murray, 1992]. That is to say, one of the cellular responses to DNA damage is cell cycle arrest. Furthermore, in the eukaryotic system, a number of studies have shown that the eukaryotic response to DNA damage includes cell cycle arrest [Weinert and Hartwell, 1988] as well as transcriptional induction [Friedberg et al., 1995]. For example, the p53 gene has been shown to be required for G1 phase arrest in human cells following treatment with ionizing radiation [Kastan et al., 1992]. In *Saccharomyces cerevisiae*, S or G2 phase arrest against DNA damage or unreplicated DNA is dependent on *RAD9*, *RAD17*, *RAD24*, *MEC1 (ESR1)*,

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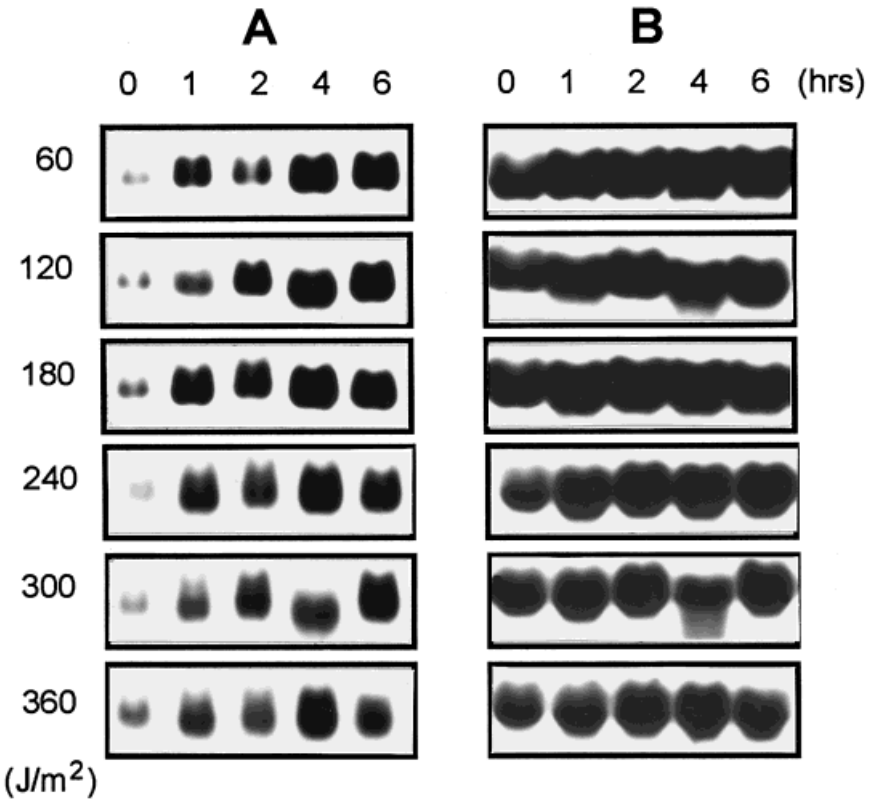

Fig. 1. Steady-state level of *uvi31*$^+$ transcript after UV irradiation. Exponentially growing *S. pombe* cells were irradiated with 60, 120, 180, 240, 300, or 360 J/m^2 of UV light, and postincubated at 30°C for 1, 2, 4, or 6 hr. Total RNA was serially isolated at each time point and Northern blot analysis was performed using the cDNA clone of *uvi31*$^+$ gene as a probe **(A).** The *act1*$^+$ gene was used for the internal control **(B).**

RAD53 (MEC2/SPK1/SAD1), or *MEC3* genes [Paulovich and Hartwell, 1995; Friedberg et al., 1995]. Additionally, in *S. pombe*, the radiation-sensitive and hydroxyurea-sensitive mutants such as *rad1, rad3, rad4 (cut5), rad9, rad17, rad21* (partial), *rad24* (partial), *rad25* (partial), *rad26*, *rad27* (chk1), and *hus1* are shown to allow cells with damaged or unreplicated DNA to enter mitosis. Other mutants, *hus6, cdc2.3w, cdc25* (overexpression), *wee1*, or *mik1* are also necessary for mitotic entry of cells with unreplicated DNA, but do not affect the ability of damaged DNA to arrest the cell cycle [Murray, 1992; Friedberg et al., 1995]. Moreover, in prokaryotes, *recA, lexA, sfiA*, and *ftsZ* genes are involved in the cell cycle control system which is a part of the SOS response [Murray, 1992].

Among UV-inducible genes previously isolated from *S. pombe* [Lee et al., 1994, 1995], *uvi31*$^+$ has many amino acid sequences similar to that of the *E. coli bolA* morphogene which is activated by *ftsZ* cell division gene and required to the morphological changes during the cell division cycle [Aldea et al., 1988, 1989]. These results suggest that the *uvi31*$^+$ gene is involved in coordinating the progress of the cell cycle after UV irradiation. This paper reports the structure and expression of *uvi31*$^+$ gene and discusses its implication for cell cycle progression.

MATERIALS AND METHODS

S. pombe Strains and Cell Culture

Haploid wild type of *S. pombe*, JY1 (*h*$^-$ 972) strain, was used for studies in gene expression. Temperature-sensitive cell cycle mutants of *S. pombe*, ED466 (*h*$^-$ *cdc10-129 leu1-32*), ED616 (*h*$^+$ *cdc22-011 leu1-32*), and Q356 (*h*$^+$ *cdc25-22 leu1-32*) strains, were used for synchronous culture analysis. *S. pombe* cells were grown in YE (3% glucose, 0.5% yeast extract) and EMM (Edinburgh minimal medium) supplemented with appropriate amino acids [Moreno et al., 1991]. Transformation of *S. pombe* cells was performed according to the lithium acetate method of Moreno et al. [1991].

UV Irradiation of *S. pombe* Cells

S. pombe cells grown to mid-log phase (OD_{595} = 0.5) were harvested, washed, and resuspended in distilled water. This cell suspension was spread onto glass petri dishes, and irradiated by a Stratalinker UV source (Stratagene) at the appropriate dose (60, 120, 180, 240, 300, or 360 J/m^2). After UV irradiation, cells were collected, resuspended in fresh medium at the original culture density, and incubated at 30°C in the dark. Aliquots of these cells were withdrawn at indicated times.

Isolation of Total RNA and Northern Hybridization Analysis

Total RNA was isolated from *S. pombe* cells as described by Jang et al. [1995]. About 40 μg of total RNA for Northern hybridization was

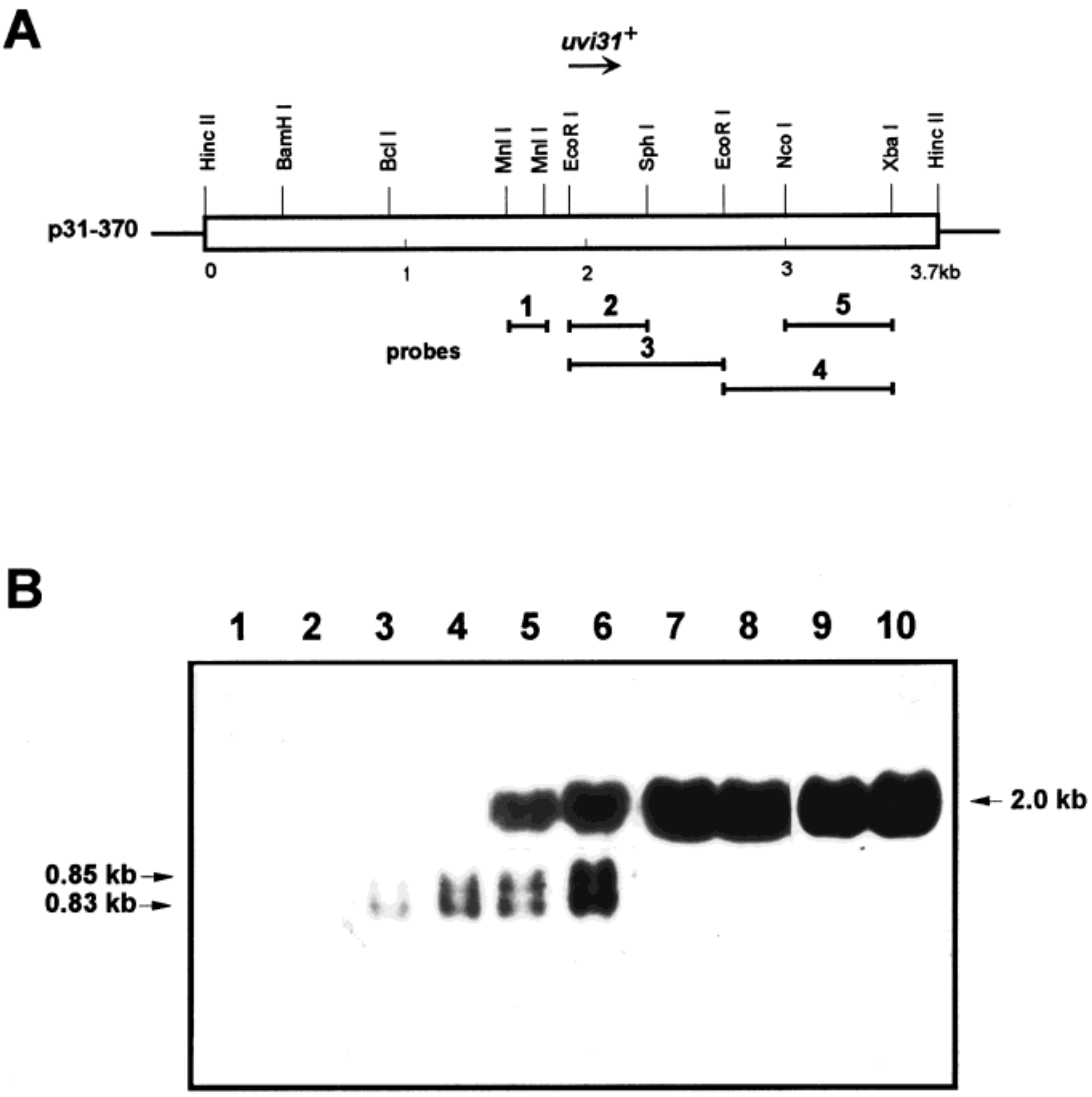

Fig. 2. Restriction map and localization of the ORF of *uvi31*$^+$ gene. **A:** Restriction map of p31–370 containing the *uvi31*$^+$ gene. The arrow indicates the ORF of the *uvi31*$^+$ gene. Numbers at the bottom of the restriction map indicate the regions of each DNA probe used for Northern blot analysis. **B:** Sublocalization of two transcripts of *uvi31*$^+$ gene using Northern blot analysis. Lanes 1, 3, 5, 7, and 9 are total RNA isolated from normal *S. pombe* cells. Lanes 2, 4, 6, 8, and 10 are total RNA isolated from UV-irradiated *S. pombe* cells (240 J/m^2, postincubation for 4 hr). Lanes 1 and 2, total RNA hybridized with probe 1 of the 225 bp *Mnl*I fragment. Lanes 3 and 4, with probe 2 of the 577 bp *Eco*RI-*Sph*I fragment. Lanes 5 and 6, with probe 3 of the 900 bp *Eco*RI fragment. Lanes 7 and 8, with probe 4 of the 778 bp *Eco*RI-*Xba*I fragment. Lanes 9 and 10, with probe 5 of the 282 bp *Nco*I-*Xba*I fragment.

electrophoresed on a 1.5% agarose gel containing formaldehyde. Northern hybridization was carried out as described by Sambrook et al. [1989], and radiolabeled DNA probes were prepared by a random priming method using [α-^{32}P]dCTP [Feinberg and Vogelstein, 1984]. The radiolabeled DNA probes were used at 42°C for 16 hr in a solution containing 50% formamide, 5 × SSC, 5 × Denhardt's solution, 0.1% SDS, and 200 μg/ml denatured salmon sperm DNA. After hybridization, blots were washed twice with 0.1 × SSC and 0.1% SDS at 65°C for 15 min, and exposed to X-ray film.

S1 Nuclease Mapping Analysis

The 5′ end of the 326 bp *Esp*I-*Rsa*I fragment was labeled with 100 μCi of [γ-^{32}P]dATP and 20 units of T4 polynucleotide kinase at 37°C for 16 hr, and separated on a 6% polyacylamide gel containing 7 M urea. The 5′ end-labeled single-stranded DNA (bottom strand) was purified as described by Sambrook et al. [1989].

About 50 μg of total RNA was mixed with 1 × 10^5 cpm of the probe DNA and hybridized in 1 × aqueous buffer (0.5 M NaCl, 1 M Tris-HCl, pH 7.5, 0.5 M EDTA) at 42°C for 16 hr. After digestion of this mixture with 200 units of S1 nuclease at 37°C for 2 hr, the size of protected DNA was analyzed by running on an 8% polyacrylamide gel containing 7 M urea.

Primer Extension Analysis

About 5 pmole of the 20mer oligonucleotide, 5′ AAAAAGAAG-AATTTCTCAAT 3′, was labeled with 100 μCi of [γ-^{32}P]dATP and 20

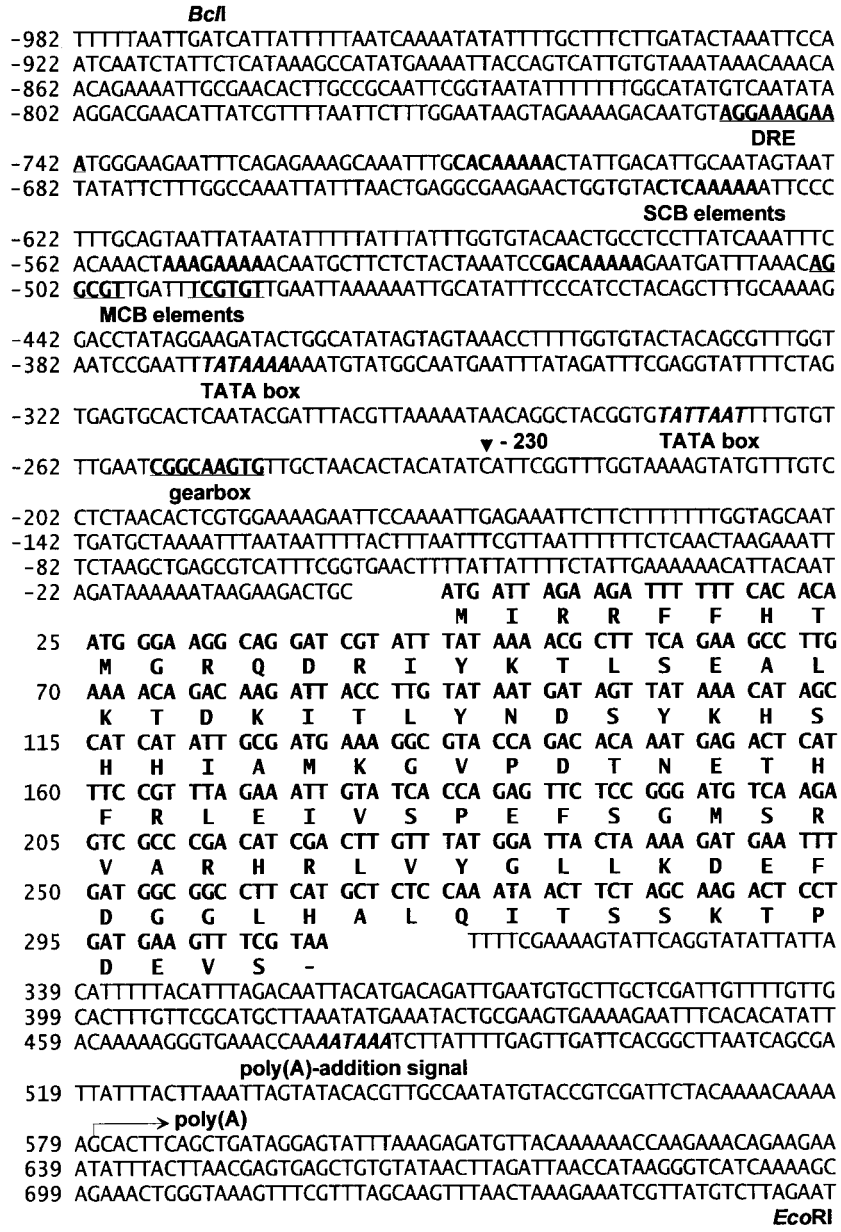

Fig. 3. Nucleotide and deduced amino acid sequences of the ORF of *uvi31*$^{+}$ gene. The putative TATA box (italic boldface), DRE (damage responsive element) (underlined boldface), MCB (*Mlu*I cell cycle box) elements (underlined boldface), SCB (SWI4/6-dependent cell cycle box) elements (boldface), gearbox element (underlined boldface), transcription start point (filled triangle), poly (A)-addition signal for mRNA synthesis (italic boldface), and poly (A)-addition site (arrow) are indicated.

units of T4 polynucleotide kinase at 37°C for 1 hr. After labeling, STE buffer (100 mM NaCl, 50 mM Tris-HCl, pH 8.0, 1 mM EDTA) was added into the labeling mixture, and unincorporated nucleotides were removed through a Nu-Clean D25 spun column (Biological Research and Imaging products, IBI).

About 1×10^5 cpm of labeled oligonucleotide was mixed with 50 μg of total RNA, and hybridized in 1 × aqueous buffer (0.5 M NaCl, 1 M Tris-HCl, pH 7.5, 0.5 M EDTA) at 42°C for 16 hr. After hybridization, this mixture was precipitated, and dissolved in cDNA synthesis buffer containing 50 units of avian myeloblastosis virus (AMV) reverse transcriptase. This extension reaction was performed at 42°C for 2 hr, and the size of synthesized cDNA was analyzed by running on an 8% polyacrylamide gel containing 7 M urea.

Synchronous Culture Analysis

Temperature-sensitive cell cycle mutants of *S. pombe*, ED466 (*cdc10-129*), ED616 (*cdc22-011*), and Q356 (*cdc25-22*) strains, were grown to mid-log phase in rich medium at the permissive temperature (25°C). These cells were collected by centrifugation, and used to reinoculate the fresh medium, which was cultured at the restrictive temperature (37°C) for 5 hr to block the progress of cell cycle, and aliquots of these cells were serially withdrawn at indicated times. The synchronized ED466 (*cdc10-129*) strain was transferred back to the permissive temperature (25°C), and aliquots of synchronized cells were serially withdrawn at indicated times.

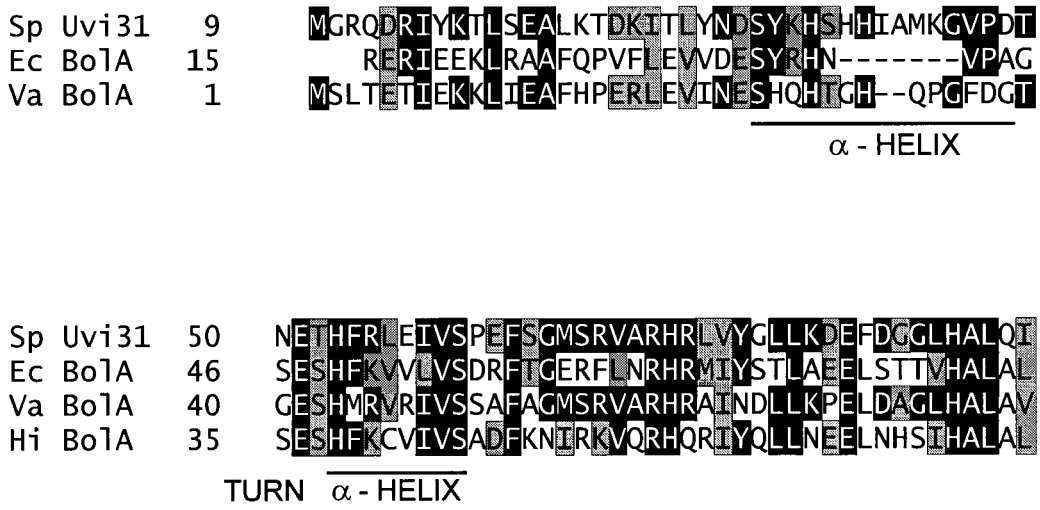

Fig. 4. Amino acid sequence alignment of *S. pombe* (Sp) Uvi31, *E. coli* (Ec) BolA, *V. alginolyticus* (Va) BolA, and *H. influenzae* (Hi) BolA proteins. Identical amino acids are emphasized by open letters with black boxes, and conservative amino acids are shown by gray boxes. The following substitutions were considered to be a similar character: D/E, N/Q, G/A, V/I/L/M, Y/F, K/R, S/T [Fankhauser et al., 1995]. The percentages of identities (similarities allowing for conservative substitution) between *S. pombe* Uvi31 and *E. coli, V. alginolyticus,* and *H. influenzae* BolA are 37 (57)%, 46 (61)%, and 40 (63)%, respectively. The underlined amino acid sequences of BolA indicate a putative DNA-binding domain.

RESULTS

Steady-State Level of *uvi31*$^+$ Transcript After UV Irradiation

A UV-inducible gene, designated as *uvi31*$^+$, has been previously isolated from the fission yeast *S. pombe* [Lee et al., 1994]. To look at the changes in the steady-state level of *uvi31*$^+$ transcript by UV irradiation, Northern blot analysis was performed. It was revealed that the level of *uvi31*$^+$ transcript is increased maximally (3.2-fold) at 4 hr after exposure to 240 J/m^2 of UV light (Fig. 1). This result suggests that the *uvi31*$^+$ gene is involved in the late events of the cellular responses to UV light.

Isolation of Genomic Clone and Localization of the ORF of *uvi31*$^+$ Gene

The genomic clone of the 3.7 kb *Hinc*II fragment containing the *uvi31*$^+$ gene was subcloned into pBluescript II KS (+) vector, and designated as p31−370. The restriction map of p31−370 is shown in Figure 2A. After subcloning and restriction mapping, Northern blot analysis was performed to locate the *uvi31*$^+$ gene within p31−370. When the cDNA probe of *uvi31*$^+$ gene hybridized with total RNA, two different transcripts of 0.83 and 0.85 kb were detected (data not shown). When five different DNA fragments within p31−370 were used for probes (Fig. 2A), probe 2 of 577 bp *Eco*RI-*Sph*I fragment was found to hybridize with both transcripts. While no signal was detected from probe 1, probe 3 hybridized not only with the 0.83 and 0.85 kb but also with the 2.0 kb transcript, and probes 4 and 5 hybridized with only the 2.0 kb transcript. Therefore, two transcripts of 0.83 and 0.85 kb were inducible to UV irradiation whereas 2.0 kb transcript was not (Fig. 2B). These data indicate that the *uvi31*$^+$ gene is located within the *Eco*RI-*Sph*I fragment of 3.7 kb DNA insert and is expressed in two transcripts of 0.83 and 0.85 kb.

Nucleotide and Amino Acid Sequence Analyses of the *uvi31*$^+$ Gene

The cDNA of the *uvi31*$^+$ gene and the 3.7 kb genomic DNA derived from p31−370 were digested with various restriction enzymes and subcloned into pBluescript SK (+) vector for nucleotide sequence determination. In the nucleotide sequence analysis using the dideoxy chain termination method [Sanger et al., 1977], the ORF of *uvi31*$^+$ gene is composed of 102 amino acids with 12 kDa in molecular mass (Fig. 3).

When this ORF was compared with a previously published database, the Uvi31 protein showed a significant homology with the bacterial BolA proteins. As shown in Figure 4, the aligned amino acid sequence of Uvi31 ORF showed 37 (57)%, 46 (61)%, and 40 (63)% identities (similarities) with *E. coli, Vibrio alginolyticus,* and *Haemophilus influenzae* BolA, respectively. Among these proteins, *E. coli* BolA was known to be involved in switching between the cell elongation and septation systems in the cell division cycle [Aldea et al., 1989]. The function of Uvi31, related to that of *E. coli* BolA, is under investigation.

To determine the transcription start point of the *uvi31*$^+$

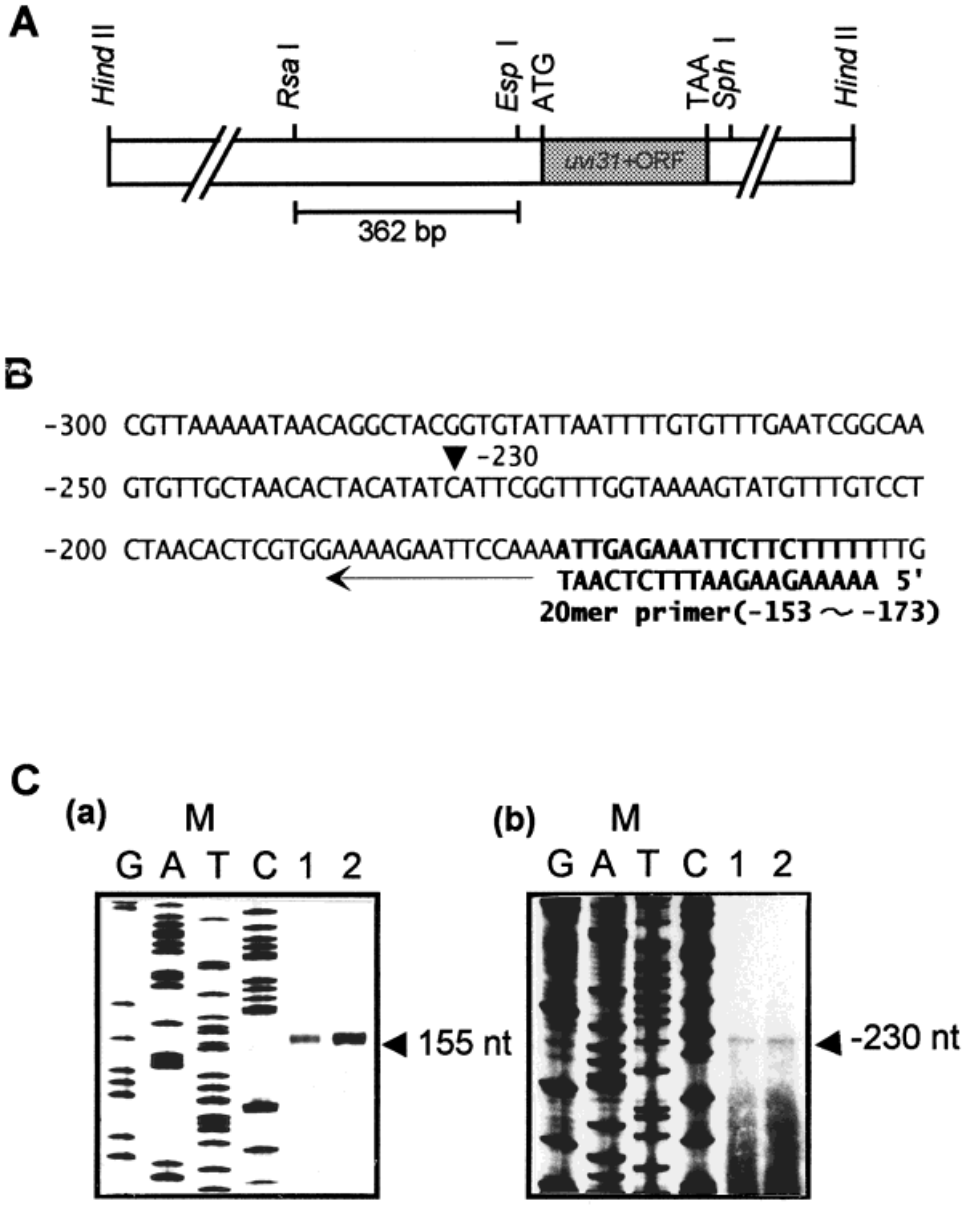

Fig. 5. Transcription start point of the *uvi31*$^+$ gene. **A:** Restriction map of *uvi31*$^+$ gene. The 362 bp *Esp*I-*Rsa*I fragment was used for S1 nuclease mapping analysis. **B:** Nucleotide sequence of the 5′ upstream region of *uvi31*$^+$ gene. Arrowhead indicates that the transcription start point is located at nt position −230 from the translation initiation codon (AUG). The synthesized 20mer oligonucleotide (5′ AAAAAGAAG-AATTTCTCAAT 3′) which was located at nt position from −153 to −173 was used for primer extension analysis. **C, a:** S1 nuclease mapping analysis. The radiolabeled single-stranded DNA probe of 362 nt was hybridized with total RNA from normal or UV-irradiated cells. After digestion with S1 nuclease, the size of protected DNA was analyzed on an 8% acrylamide gel containing 7 M urea. Lane M, nucleotide sequence of M13mp18 as a size marker; lane 1, normal cells; lane 2, UV-irradiated cells (240 J/m^2, postincubation for 4 hr). **C, b:** Primer extension analysis. The radiolabeled primer of 20mer oligonucleotide was hybridized with total RNA from normal or UV-irradiated cells. After extension reaction with AMV reverse transcriptase, the size of synthesized cDNA was analyzed on an 8% acrylamide gel containing 7 M urea. Lanes are the same as those in **a**.

gene, S1 nuclease mapping and primer extension analyses were performed (Fig. 5). These experiments showed that the transcription of the *uvi31*$^+$ gene starts at nt position −230 from the translation initiation codon (AUG) (Fig. 5B and C). Also, in the 3′ end sequence of its cDNA clone, the putative poly (A)-addition signal AAUAAA and the poly (A)-addition site are located at nt positions 478 and 580, respectively (Fig. 3). Hence, the production of two transcripts of 0.83 and 0.85 kb may be attributed to different lengths in poly (A) tail or an unidentified cDNA clone.

The upstream sequence of the *uvi31*$^+$ gene contains

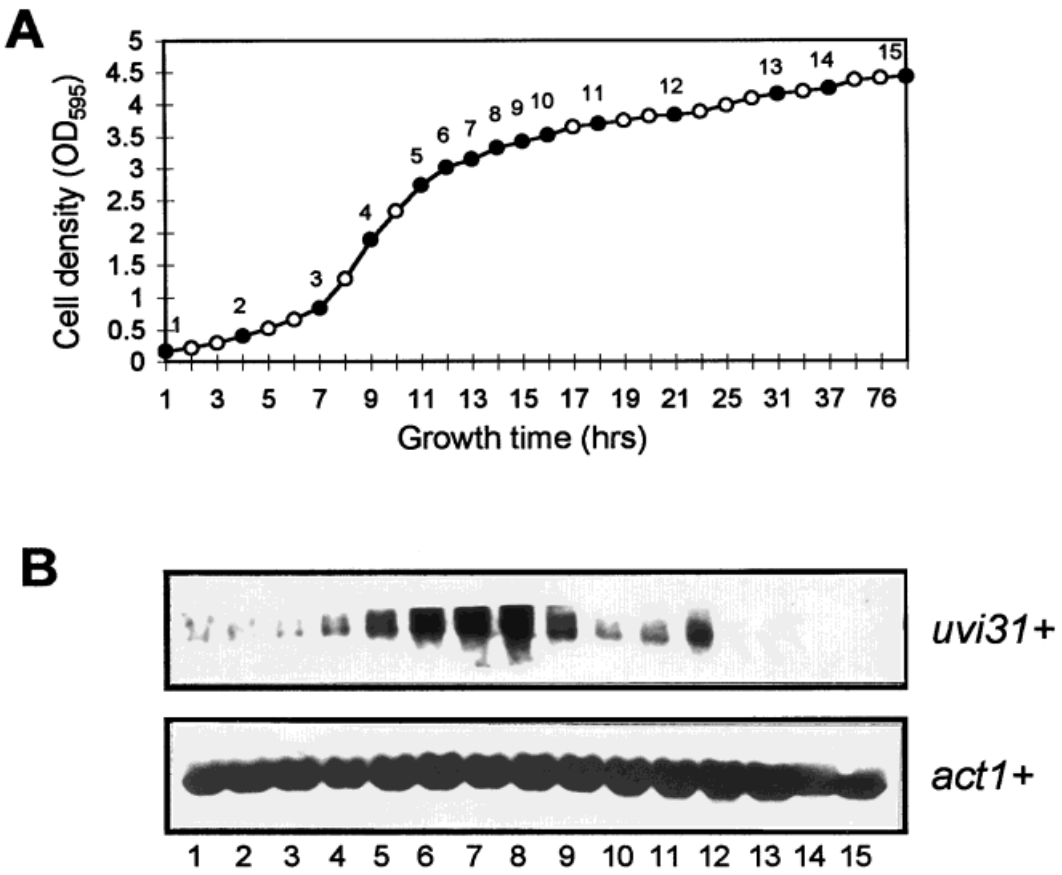

Fig. 6. Growth phase–dependent expression of *uvi31*$^+$ during cellular growth. **A:** Growth curve from the experimental culture. Numbers indicate the time points at which cells were collected for the isolation of total RNA. **B:** Steady-state level of *uvi31*$^+$ transcript during the growth to stationary phase. Northern blot analysis was carried out using probes for *uvi31*$^+$ and *act1*$^+$ genes. Lanes correspond to the numbered points in A.

two putative TATA boxes, TATTAAT and TATAAAA, at nt positions −276 and −372, respectively. Additionally, there are two putative *Mlu*I cell cycle box (MCB) elements, TCGTGT and AGGCGT, present with one and two mismatches to *S. cerevisiae* MCB element (ACGCGT) at nt positions −493 and −504, respectively. Interestingly, four putative SWI4/6-dependent cell cycle box (SCB) elements, GACAAAAA, AAAGAAAA, CTCAAAAA, and CACAAAAA, are also present in the vicinity of two putative MCB elements with two mismatches to *S. cerevisiae* SCB element (CACGAAAA) (Fig. 3). In *S. cerevisiae*, several genes which contain the MCB and SCB elements in the promoter regions were periodically expressed in late G1 phase [Johnston, 1992]. Moreover, in *S. pombe*, the transcription of genes such as *cdc22*$^+$ [Gordon and Fantes, 1986], *cdc18*$^+$ [Kelly et al., 1993], *cdc15*$^+$ [Fankhauser et al., 1995], and *rhp51*$^+$ [Jang et al., 1994, 1996] which contain the MCB and SCB elements were periodic in their cell cycles reaching a maximum near G1 or G1/S boundary, but involved a more restricted set of genes than in *S. cerevisiae*. Furthermore, the putative damage responsive element (DRE) (AGGAAAGAAA) and gearbox element (CGGCAAGTG) are also found in the upstream region of *uvi31*$^+$ gene (Fig. 3). The 'DRE' element was the sequence shared in the upstream region of several DNA damage inducible genes [Wolter et al., 1996; Friedberg et al., 1995], and the gearbox element was essential for growth phase–dependent expression of *ftsZ*-dependent *bolA* morphogene and *ftsQAZ* cell division genes of *E. coli* [Aldea et al., 1990]. Finally, these putative promoter elements provide a possibility of the cell cycle–regulated, DNA damage–inducible, and growth phase–dependent expression of *uvi31*$^+$ gene.

Growth Phase–Dependent Expression of the *uvi31*$^+$ Gene During Cellular Growth

As previously noted, the putative gearbox element, an essential sequence for growth phase–dependent expression of *E. coli* cell division genes, was found in the upstream region of the *uvi31*$^+$ gene. Therefore, to look at how the transcription of *uvi31*$^+$ is regulated during the

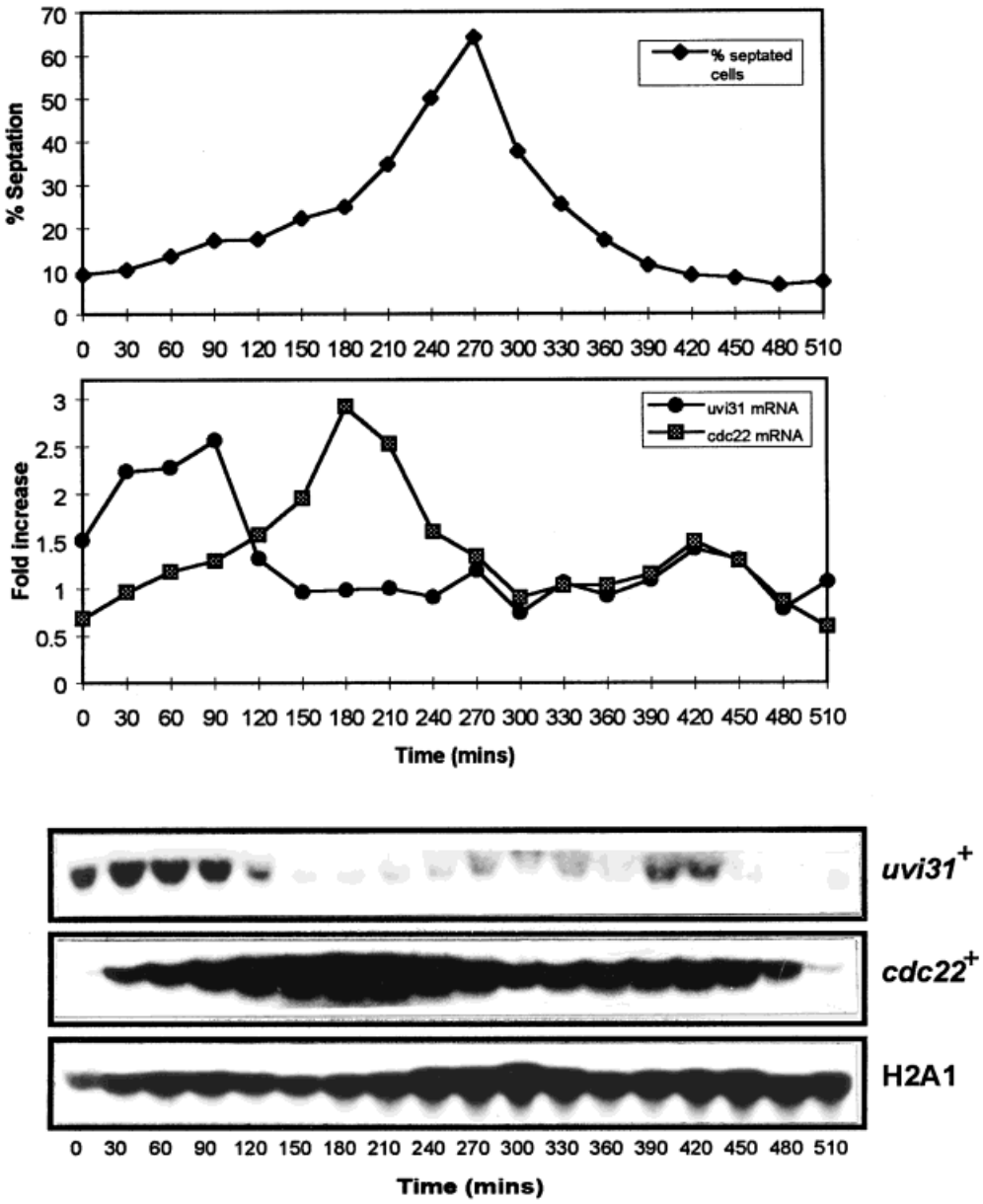

Fig. 7. Periodicity of *uvi31*$^+$ transcript during the cell cycle. Changes in the steady-state levels of *uvi31*$^+$ mRNA during the cell cycle were analyzed in a synchronized population of *cdc10-129* cells. The *cdc10-129* cells were synchronized by temperature block (37°C), and synchronized cells were transferred back to the permissive temperature (25°C). Samples were serially taken for the microscopic examination of cell synchrony and the isolation of total RNA at each time point. Cell synchrony, which is shown in the top panel, was examined by determining the percentage of cells with visible septa at each time point. Northern blot analysis was performed using the probes for *uvi31*$^+$, *cdc22*$^+$, and histone H2A1. Relative amounts of *uvi31*$^+$ and *cdc22*$^+$ mRNA compared with histone H2A1 mRNA are shown above the blots. The *cdc22*$^+$ gene was used for the control of periodic expression, whereas the histone H2A1 gene was used for the control of constitutive expression during the cell cycle [Gordon and Fantes, 1986; Matsumoto et al., 1987].

cellular growth, Northern blot analysis was performed. It was shown that the level of *uvi31*$^+$ transcript is transiently increased more than twofold when cells reach the diauxic shift phase, and is rapidly decreased as cells approach the stationary phase (Fig. 6). These data indicate that *uvi31*$^+$ transcription is indeed regulated according to the growth phase during this cellular growth.

Periodic Expression of the *uvi31*$^+$ Gene During the Cell Cycle

As mentioned previously, since the *uvi31*$^+$ gene contained the putative MCB and SCB elements in its promoter region, it is interesting to investigate the expression pattern of the *uvi31*$^+$ gene during the cell cycle. Total

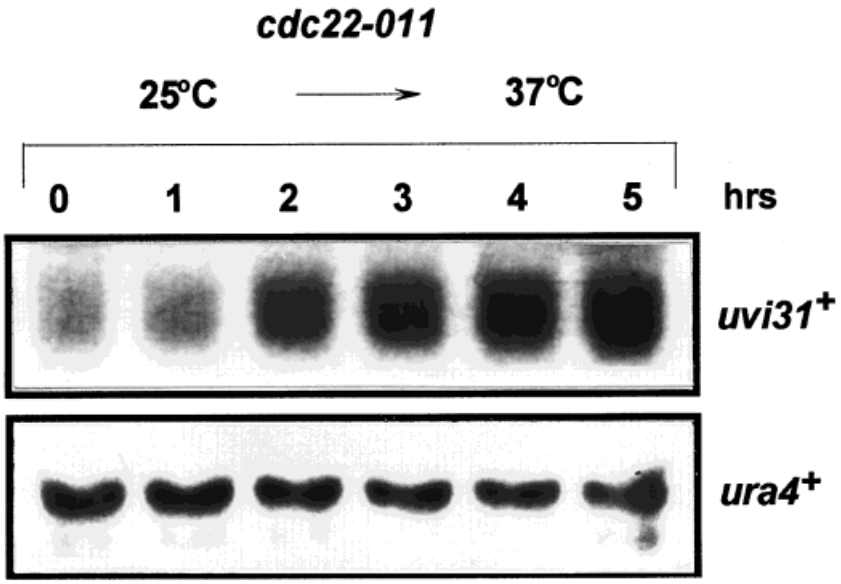

Fig. 8. Level of *uvi31*$^+$ mRNA after S phase arrest. The level of *uvi31*$^+$ transcript was followed by Northern blot analysis after shifting *cdc22-011* cells from the permissive (25°C) to the restrictive (37°C) temperature. Total RNA was serially isolated from *cdc22-011* cells undergoing arrest in S phase. The number of hours after the temperature shift are given at the top. The *ura4*$^+$ gene was used for the internal control.

RNA for Northern hybridization was prepared from synchronous culture of the ED466 (*cdc10-129*) strain, a temperature sensitive cell cycle mutant, maintained at 25°C for one generation after synchronization at 37°C for 5 hr. In this experiment, the level of *uvi31*$^+$ transcript rose to a peak in G1 phase before the level of *cdc22*$^+$ transcript reached its peak in the G1/S boundary of the cell cycle (Fig. 7). These results showed that the *uvi31*$^+$ gene is indeed expressed periodically during the cell cycle and suggest that the Uvi31 protein plays a role in G1 or G1/S phases.

Level of *uvi31*$^+$ Transcript Increases After S Phase Arrest

To confirm the periodic expression of the *uvi31*$^+$ gene, it is worthwhile to examine whether the *uvi31*$^+$ transcript increases after cell cycle arrest at one of three checkpoints. Total RNA for Northern blot analysis was taken from a synchronous culture at the nonpermissive temperature (37°C) of ED466 (*cdc10-129*), ED616 (*cdc22-011*), and Q356 (*cdc25-22*) strains undergoing arrest in G1, S, and G2 phase, respectively. In *cdc22-011* cells, there is an increase in the level of *uvi31*$^+$ transcript after S phase arrest (Fig. 8). However, in *cdc10-129* and *cdc25-22* cells, its transcript level did not increase (data not shown). These results indicate that *uvi31*$^+$ transcription is regulated during the cell cycle and suggest that the *uvi31*$^+$ gene is involved in cell cycle control after DNA replication checkpoint arrest.

DISCUSSION

The level of *uvi31*$^+$ transcript is increased maximally (3.2-fold) at 4 hr after exposure to 240 J/m^2 of UV light. Also, this gene contains a putative 'DRE' element in its promoter region, which is shared by many DNA damage–inducible *S. cerevisiae* genes [Wolter et al., 1996; Freidberg et al., 1995].

The *uvi31*$^+$ gene encodes a protein of about 12 kDa in molecular mass from the single open reading frame (ORF) with 102 amino acids in length. In the comparison with previously published sequences, the predicted amino acid sequence of this gene showed a significant homology with the bacterial BolA proteins. Among these proteins, *E. coli* BolA is known to be involved in the morphological changes during the cell division cycle [Murray, 1992]. Additionally, BolA acts as an inducing factor for the synthesis of cell envelope in the transition to the stationary phase during cellular growth [Aldea et al., 1989]. This inducible function of BolA may be caused by its putative DNA-binding domain which is also found in Uvi31. Moreover, the *uvi31*$^+$ gene contains the putative gearbox element in its promoter region. This element was essential for the growth phase–dependent expression during cellular growth in *E. coli ftsQAZ* cell division genes and *ftsZ*-dependent *bolA* morphogene [Aldea et al., 1990]. In fact, the transcript level of the *uvi31*$^+$ gene is maximally increased when cells reach the diauxic shift phase just before stationary phase during cellular growth.

The most interesting feature about the *uvi31*$^+$ gene is its periodic expression during the cell division cycle. Fur-

thermore, the promoter region of *uvi31*$^+$ contains two putative MCB elements and four putative SCB elements, which are required for cell cycle–regulated expression in late G1 phase during the cell cycle in *S. cerevisiae,* as well as in *S. pombe* [Johnston, 1992]. Recently, there is increasing evidence that periodic gene expression plays a crucial role in cell cycle control [Johnston, 1992]. A number of DNA damage–responsive and DNA synthesis–responsive cell cycle control genes have been identified. Among these genes, mRNA and protein levels of several genes were increased after cell cycle arrest [Kovelman and Russell, 1996]. The level of *uvi31*$^+$ mRNA is also increased after S phase arrest.

Although the roles of DRE, MCB, SCB, and gearbox elements in the regulation of *uvi31*$^+$ gene expression have not yet been tested, as discussed above, these elements are thought to provide inducibility by UV irradiation, periodicity during the cell cycle, and growth phase–dependent inducibility during cellular growth. Altogether, these observations suggest a possibility that the *uvi31*$^+$ gene is required to coordinate the progress of the cell cycle in late events (cell cycle control after DNA replication checkpoint arrest) after DNA damage (UV induced). The direct role of the *uvi31*$^+$ gene product in cellular processes may be revealed by several approaches in further studies.

ACKNOWLEDGMENTS

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REFERENCES

Aldea M, Hernandez-Chico C, de la Campa AG, Kushner SR, Vicente M (1988): Identification, cloning, and expression of *bolA*, an *ftsZ*-dependent morphogene of *Escherichia coli.* J Bacteriol 170:5169–5176.

Aldea M, Garrido T, Hernandez-Chico C, Vicente M, Kushner SR (1989): Induction of a growth-phase-dependent promoter triggers transcription of *bolA*, an *Escherichia coli* morphogene. EMBO J 8:3923–3931.

Aldea M, Garrido T, Pla J, Vicente M (1990): Division genes in *Escherichia coli* are expressed coordinately to cell septum requirements by gearbox promoters. EMBO J 9:3787–3794.

Carr AM, Hoekstra MF (1995): The cellular responses to DNA damage. Trends Cell Biol 5:32–40.

Fankhauser C, Reymond A, Cerutti L, Utzig S, Hofmann K, Simanis V (1995): The *S. pombe cdc15* gene is a key element in the reorganization of F-actin at mitosis. Cell 82:435–444.

Feinberg AP, Vogelstein B (1984): A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137:266–267.

Friedberg EC, Walker GC, Siede W (1995): ''DNA Repair and Mutagenesis.'' Washington, D.C.: ASM Press, pp 595–631.

Gordon CB, Fantes PA (1986): The *cdc22* gene of *Schizosaccharomyces pombe* encodes a cell cycle-regulated transcript. EMBO J 5:2981–2985.

Jang YK, Jin YH, Kim EM, Fabre F, Hong SH, Park SD (1994): Cloning and sequence analysis of *rhp51*$^+$, a *Schizosaccharomyces pombe* homolog of the *Saccharomyces cerevisiae RAD51* gene. Gene 142:207–211.

Jang YK, Jin YH, Kim MJ, Seong RH, Hong SH, Park SD (1995): A simple and efficient method for the isolation of total RNA from fission yeast *Schizosaccharomyces pombe.* Biochem Mol Biol Int 37:339–344.

Jang YK, Jin YH, Myung K, Seong RH, Hong SH, Park SD (1996): Differential expression of the *rhp51*$^+$ gene, a *recA* and *RAD51* homolog from the fission yeast *Schizosaccharomyces pombe.* Gene 169:125–130.

Johnston LH (1992): Cell cycle control of gene expression in yeast. Trends Cell Biol 2:353–357.

Kastan MB, Zhan Q, El-Deiry WS, Carrier F, Jacks T, Walsh WV, Plunkett BS, Volgelstein B, Fornace AJ Jr (1992): A mammalian cell cycle checkpoint pathway utilizing p53 and *GADD45* is defective in Ataxia-Telangiectasia. Cell 71:587–597.

Kelly TJ, Martin GS, Forsburg SL, Stephen RJ, Russo A, Nurse P (1993): The fission yeast *cdc18*$^+$ gene product couples S phase to START and mitosis. Cell 74:371–382.

Kovelman R, Russell P (1996): Stockpiling of Cdc25 during a DNA replication checkpoint arrest in *Schizosaccharomyces pombe.* Mol Cell Biol 16:86–93.

Lee JK, Park EJ, Chung HK, Hong SH, Joe CO, Park SD (1994): Isolation of UV-inducible transcripts from *Schizosaccharomyces pombe.* Biochem Biophys Res Commun 202:1113–1119.

Lee JK, Kim M, Choe J, Seong RH, Hong SH, Park SD (1995): Characterization of *uvi15*$^+$, a stress-inducible gene from *Schizosaccharomyces pombe.* Mol Gen Genet 246:663–670.

Matsumoto S, Yanagida M, Nurse P (1987): Histone transcription in cell cycle mutants of fission yeast. EMBO J 6:1093–1097.

Moreno S, Klar A, Nurse P (1991): Guide to yeast genetics and molecular biology. In Guthrie C, Fink GR (eds): ''Molecular Genetic Analysis of Fission Yeast *Schizosaccharomyces Pombe.*'' Methods Enzymol 194:795–823.

Murray AW (1992): Creative blocks: Cell cycle checkpoints and feedback controls. Nature 359:599–604.

Paulovich AG, Hartwell LH (1995): A checkpoint regulates the rate of progression through S phase in *S. cerevisiae* in response to DNA damage. Cell 82:841–847.

Sambrook J, Fritsch EF, Maniatis T (1989): ''Molecular Cloning: A Laboratory Manual,'' 2nd ed. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Sanger F, Nicklen S, Coulson AR (1977): DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467.

Weinert TA, Hartwell LH (1988): The *RAD9* gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae.* Science 241:317–322.

Wolter R, Siede W, Brendel M (1996): Regulation of *SNM1*, an inducible *Saccharomyces cerevisiae* gene required for repair of DNA cross-links. Mol Gen Genet 250:162–168.

Zhou Z, Elledge SJ (1993): *DUN1* encodes a protein kinase that controls the DNA damage response in yeast. Cell 75:1119–1127.

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