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 *Mlul* cell cycle box (MCB), SW14/6-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/m². 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 (1997 Wiley-Liss, Inc.

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

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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/m² 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 (OD₅₉₅ = 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/m²). 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 μ g 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/m², postincubation for 4 hr). Lanes 1 and 2, total RNA hybridized with probe 1 of the 225 bp *MnI*I fragment. Lanes 3 and 4, with probe 2 of the 577 bp *Eco*RI-*SphI* 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-*XbaI* fragment. Lanes 9 and 10, with probe 5 of the 282 bp *NcoI-XbaI* 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 -³²P]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 *EspI-RsaI* fragment was labeled with 100 μ Ci of [γ -³²P]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' AAAAAGAAGAAGAATTTCTCAAT 3', was labeled with 100 μ Ci of [γ -³²P]dATP and 20

Bcl

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-862 ACGAGAAATTCGCGAACACTTGCCGCAATTCGGTTATATTTTTTTGCATTATATTAT -802 AGGACGAACATTATCGTTTTAATTCTTTGGAATAAGTAGAAAAGACAATGTAGGAAAAGA -802 AGGACGAACATTATCGGTTTAATTCGTTGGAATAAGTAGAAAAGACAATGTAGGAAAAGAA -742 ATGGGAAGAATTTCGAGAGAAGCAAATTTGCACAAAAACTATTGACATTGCAATAGAAT -822 TTTGCAGTAATTATTATATTTTTATTTGACGTGGGCGAAGAACTGGTGGACTCAAAATTCCC -622 TTTGCAGTAATTATAATATTTTTATTTGACTTGGGTGACACAGCGCCTCCTTATACAATTCC -622 TTTGCAGTAATTATAATATTTTTATTTGGTGTGACAACTGCCTCCTTATACAATGC -622 TTTGCAGTAATTATAATATTTTTATTTGGCATTAAATTGGATATTTCCCATCCTACAGCTTTGCAAAAAG -622 TTGCAGTAATTGGAAAACAATGCTTCTCACAAAATGCAACTGCCTCCTACAGCTTTGGAAAAG -622 TTGCAGTGGAAAAACAATGGCAATAGTAGTAGTAATACGAGTTTTGGTGTACTACAGGGTATTTATAAAAAA -424 GACCTATAGGAAAGAATGGCAATTAGTAGTAAAAAAACAGGCTACGGTGTATTAAGAAG -425 TGGAGCCACCAATAGGATTACGGCAATGAATTGAGAATTTCCAAGGTTTGGT -422 TGCAACCTGGGAAAAACAATTGCATCACATACGTTGGTGACAAAGGATGTTTGGT -220 TTCTAAACATGGGCAAAGGATTCCAAAAATGGAAATTCGATGTTGGTGAAAAGGATGTTTGGT -220 TTCTAAACCTGGGGAAAAGAATTCCAAAATTGGAAAATTCGAGAATTCTTCTTTTTTGGTAGCAAT -22 TGCAACCGGGCAAAGGAATTCCAAAATTGGAAAATTCGAAAAGCATTGACAAT -22 TGCAACCTGGGGAAAAGAATTCCAAAATTGGAAAATTGGTAGAAAAGTATGCAAT -22 TGCAACCGGGGAAAGCAATTCCAAAATTGGAAAATTCCAAAAACATTAACAAT -22 TGCAACCGGGGAAAGCAATTCCAAAATTGGAAAATTCTTCTTTTTTTGGTAGCAAAT -22 AGATAAAAAATAAGAAGACTGC ATG ATT ATA AAA AGC GTT TCA GAA GCC TTG M G R Q D R I Y K T L S E A L 70 AAA ACA GAC AAG ATT ACC TTG TAT AAA AGC TTT CA GAA GCC TTG M G G R Q D R I Y K T L S E A L 70 AAA ACA GAC AAG ATT ACC TTG TAT AAA AGC ACA AAT GAG ACC TAT H H I A M K G C V P D T N E T H 160 TTC CGT TTA GGA ATG CGT GCT TCT CAA ATA AGT AGA ACC TCA H H I A M K G C V P D T N E T H 170 AA ACA GAC CAAC AAT ATG CTT GT TAT GAA AGA TTACACATATT V A R H H R L V Y G L L K D E F 250 GAT GAC GC CC CC CAA CTA GCT GTT CTC CAA ATA ACT TCT ACC AAG ACT 171 V A R H H R L V Y G L L K D E F 250 GAT GAC GC CC CC CT CAT CCT GCT CTC CAA ATA ACT TCT CCAAAAGGAATTTACACACATATT V A R H H R L V Y G L L K D E F 250 GAT GAA GTT TCG TAAAATTACTGACAGATGTACAAAAGAATTTACACACAAAA F R L E E I V S -	-922	ATCAA	ТСТА	TTC	ICA I A	AAGC	CAT?	ATGAA		ACC	AGICA	ALICI	GTAA	ATAA	ACAA	ACA
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MCB elements-442GACCTATAGGAAGATACTGGCATATAGTAGTAGTAGAACCTTTTGTGTGTACTACAGCGTTTGGT-382AATCCGAATTTAAGAAAATGTATGGCAATGGAATTTATAGATTTCGAGGTATTTATAGGTTTTCTAG TATA box-322TGAGTGCACTCAATACGATTTACGTTAAAAATGGCAATGAACTAGCGGCGGCGGTGTATTAATTTTGTGT $y \cdot 230$ -262TTGAATCGGCAAGGIGTTGCTAACACTACATACATCACTACGTTCGGTAAAAATGGAAATTGGTAAAAATGGAAATTGGTAAAAATGGAAATTTGGTAAAAATTTTGTTTGGTAGAAAAT -202-202CTCTAACACTCGTGGAAAAGAATTCCAAAATTGAGAAATTGGGTAAATTTTGGTAGCAAT -142-203TATA box-204CTCTAACACTCGTGGAAAAGAATTCCAAAATTGAGAAATTCTGTTTTTTTGGTAGCAAT -202-205CTCTAACACTCGTGGAAAAGAATTCCAAAATTGAGAAATTTGGTAAAATTTTGGTAGCAAT -22-202CTCTAACACTCGTGGAAAAGAATTCCAAAATTGAACATTTACTTTTATTTTTTGTTGGAACAAAAAAATAAGAAGACAGC AAGAGACACACACACAACAAATTAACATTTAATTTTGGTGAACAATTTACAAT -22-202CTCTAACACTCGTGGAAAAGAATTCGGTGAACTTTTAATTTTTTTT	-502	GCGTT	GATI	TCG	GII	JAATI	AAAA	VAAT	GCA	ATT	ICCCA	AICCI	ACAG	CLU	GCAA	AAG
-442 GACCTATAGGAAGATACTGGCATATAGTAGTAGTAGTAAACCTTTTGGTGTACTACAGGGTTTGGT -382 AATCCGAATTATAAAAAATTGTATGGCAATGAATTTATAGATTTCGAGGTATTTATATGTTTGTG TATA box -322 TGAGTGCACTCAATACGATTTACGTTAAAAATAACAGGCTACGGTGATTTAATTTTGTGT		MC	B ele	ment	S											
 -382 AATCCGAATT<i>TATAAAA</i>AAATGTATGGCAATGAATTTATAGATTTCGAGGTATTTTCTAG TATA box -322 TGAGTGCACTCAATAGCATTTACGTTAAAAATAACAGGCTACGGTG<i>TATTAAT</i>TTTGTGT y - 230 TATA box -262 TTGAATCGGCAACTGTTGCTAACACTACATTACATTCGTTTGGTAGAAGTATGTTTGTC gearbox -202 CTCTAACACTCGTGGAAAAGAATTCCAAAATTGAGAAATTCTTCTTTTTTGGTAGCAAT -142 TGATGCTAAAAATTAATAATTTTACTTTAATTTTCGTTAATTTTTTTGGTAGCAAT -22 AGATAAAAAATTAAAGAAGACTGC ATG ATT AGA AGA TTT TAT AAAAAACATAACATACATT 25 ATG GGA AGG CAG GAT CGT ATT TAT AAA ACG CTT TCA GAA GCC TTG M G R Q D R I Y K T L S E A L 70 AAA ACA GAC AAG ATT ACC TTG TAT AAA GAT AGT TAT AAA CAT AGC K T D K I T L Y N D S Y K H S 115 CAT CAT ATT GCG ATG ATG ATT CAC ACA GAG ACT CAT H H I A M K G V P D T N E T H 160 TTC CGT TTA GAA ATT GTA TCA CCA GAG TTC TCC GGG ATG TCA AGA F R L E I V S P E F S G M S R 205 GTC GCC CGA CAT CGA CTT GTT TAT GGA TTA CTA AAG AGA ACT ATT A A A ACA GAC ATT GCT CTC CAA ATA ACT TCT AGC AAG ACT CAT H H I A A K L V Y G L L K D E F 250 GAT GGC GGC CTT CAT GCT CTC CAA ATA ACT TCT AGC AAG ACT CCT D G G L H A L Q I T S S K T P 295 GAT GGC GGC CAT CAACTTACATTACATACAGCAATTTCACGCTAAAAAGGTGTTAAAATGGCTTAAAATGAACAATTAAATTCAATTAAATTGCAATAATTCAAAAAGGATGTTCACGCACTAATTAAT	-442	GACCT	ATAC	GAAG	GATAC	TGGC	ATA1	AGT A	\GTA/	ACC	пп	GTGT	ACTA	CAGO	GTTT	GGT
TATA box -322 TGAGTGCACTCAATACGATTTACGTTAAAATAACAGGCTACGGTGTATTAATTTTGTGT v - 230 TATA box -262 TTGAATCGGCAAGTGTTGCTAACACTACATTACATTCGTTGGTAGCAATGTTGGTAGCAATGGTGTGCTAACACTCGTGGCAAAAGGAATTCCCAAAATTGGAGAAATTCTTCTTTTTTTGGTAGCAAATT -202 CTCTAACACTCGTGGGAAAAGAATTCCCAAAATTGAGAAATTCTTCTTTTTTTT	-382	AATCO	GAAT	TTA	ГААА		GTAT	rggc/	ATG/	ATT	ΓΑΤΑΟ	ATT	CGAG	GTAT	ттс	TAG
-322 TGAGTGCACTCAATACGATTTACGTTAAAAATAACAGGCTACGGTG <i>TATTAAT</i> TTTGTGT v - 230 TATA box -262 TTGAAT <u>CGGCAAGTG</u> TTGCTAACACTACATATCATTCGGTTTGGTAAAAGTATGTTTGTC gearbox -202 CTCTAACACTCGTGGAAAAGAATTCCCAAAATTGAGAAATTCTTCTTTTTTTGGTAGCAAT -142 TGATGCTAAAATTTAATATTTTACTTTAATTTGGTAATTTTTCTCAACTAAGAAAATT -22 AGATAAAAAATAAGAAGACTGC ATG ATT TATATATTTTCTATTGAAAAAAACATTACAAT -22 AGATAAAAAATAAGAAGACTGC ATG ATT TAT AAA ACG CTT TCA GAA GCC TTG M G R Q D R I Y K T L S E A L 70 AAA ACA GAC AAG ATT ACC TTG TAT AAT GAT AGT TAT AAA CAT AGC K T D K I T L Y N D S Y K H S 115 CAT CAT ATT GCG ATG AAA GCC GTA CCA GAC ACA AAT GAG ACT CAT H H I A M K G V P D T N E T H 160 TTC CGT TTA GAA ATT GTA TCA CCA GAG TTC TCC GGG ATG TCA AGA F R L E I V S P E F S G M S R 205 GTC GCC CGA CAT CGA CTT GTT TAT GGA TTA CTA AAA GT GAA TTT V A R H R L V Y Y G L L K D E F 250 GAT GGC GGC CTT CAT GCT CTC CAA ATA ACT TCT AGC AAGA ACT CCT D G G L H A L Q I T S S K T P 295 GAT GGC GGC CTT CAT GCT CTC CAAATTGGTATTCAGGTATATTATA 39 CATTTTACATTTAGAAATTACATGACAGATTGACAGATTCAGCGAAGTATTCAGGTATATTATAAA 59 ACAAAAAGGGTGGCAGATTTAAAATAGCAAATACTGCCAAAAGAAATTCAGCGTTAATTATTA 59 CACTTGTTCGCATGCTTAAATTACAATTAGCAATTGGCAAGTGTAATTCAGGTATATTATAA 519 TTATTTACATTTAGAAATTACAATTACAAGAGTGGCTTGCCCGATTCTACAAAACAAAA F R L F I C Y S - 339 CATTTTACATTTAGAAATTACAATTACAAGAGAATTGGCAAGGAATTTCAGCGATTATTATTA D E V S - 339 CATTTTACATTTAGAAATTACAATTACAAGAGATTGGCATGGCTTGCTCGATTGTTTTGTTG 399 CACCTTAAAATTAAATTACAACTTACATGACAAGAATTCCACCGCTTAAAACAAAA $\longrightarrow poly(A)$ -addition signal 519 TTATTTACTTAACTTAAACTAGGAGGTGTTACAAAAAAACCAAAAACAAAAACAAAAAACAAAAACAAAA	TATA box															
 Y - 230 TATA box Y TATA CTTAGCTAAAAGTATGCAAAAGAAATTGCAAAAAAAAAA	222	тсаст				^^ ^ TTT				\ACA	CCT	ACCC1	CTAT	TAAT		тст
 262 TTGAATCGGCAAGTGTTGCTAACATGACTACCATATCATTCGGTTTGGTAAAAGTATGTTTGTC gearbox 202 CTCTAACACTCGTGGAAAAGAATTCCAAAATTGAGAAATTCTTCGTTTTTTTGGTAGCAAT 2142 TGATGCTAAAATTTAATAATTTTACTTTAATTTAATTTTCGTTAATTTTTTTCGATAGAAAACATTAGAAAT 22 AGATAAAAAATAAAGAGACTGC ATG ATT AGA AGA TTT TAT CAC ACA M I R R F F H T 25 ATG GGA AGG CAG GAT CGT ATT TAT AAA ACG CTT TCA GAA GCC TTG M G R Q D R I Y K T L S E A L 70 AAA ACA GAC AAG ATT ACC TTG TAT AAT GAT AGT TAT AAA CCT TAGC K T D K I T L Y N D S Y K H S 115 CAT CAT ATT GCG ATG AAA GGC GTA CCA GAC ACA AAT GAG ACT CAT H H I A M K G V P D T N E T H 160 TTC CGT TTA GAA ATT GTA TCA CCA GAG TTC TCC GGG ATG TCA AGA F R L E I V S P E F S G M S R 205 GTC GCC CGA CAT CGA CTT GTT TAT GGA TTA CTA AAG GAT GAA TTT V A R H R L V Y G L L K D E F 250 GAT GGC GGC CTT CAT GCT CTC CAA ATA ACT TCT AGC AAG ACT CCT D G G L H A L Q I T S S K T P 295 GAT GGC GGC AGT TAAATGACATTACAATACTGCAAAGAATTCAGGTAATTATTATA D E V S - 399 CATTTTACTTAGAAATTAGAATACCGAAGAATTCAAGAATTCAGGTAAATTAAAAGGAATTCAAGCAATTACAAAAAGGAATTACAAAAAGGAATTACAAAAAGGAATTACAACAAAAAAGGAATTACAACAAAAAGGAATTACAACAAAAAAGGAATTACAACAAAAAAGGAATTACAACAAAAAAGGAATTACAACAAAAAGGAATTACAACAAAAAAGGAATTACAACAAAAAAAA	-322	IGAGI	GCAC	.100	ATACC	ATT	ACU	11/0/0	~~ !/	NUN			TA:			101
 -262 TTGAATCGGCAACTGTTGCTAACACTACATATCATTCGGTTTGGTTGG										7 - 23	0)X	
$\begin{array}{c} \mbox \\ -202 & CTCTAACACTCGTGGAAAAGAATTCCAAAATTGAGAAATTCTTCTTTTTTTGGTAGCAAT -142 & TGATGCTAAAATTTAATAATTTTACTTTAATTTCGTTAATTTTTTTT$	-262	TIGAA		JAAO	JGI	IGCTA		LIACA	ATAT	ALIC	-661	1661	АААА	GIAI	GIII	GIC
-202 CTCTAACACTCGTGGAAAAGAATTCCAAAATTGAGAAATTCTTCTTTTTTTGGTAGCAAT -142 TGATGCTAAAATTTAATAATTTTACTTTAATTTTCGTTAATTTTTTCTAATTGAAAAACATTAGCAAT -22 AGATAAAAAATAAGAAGACTGC ATG ATT TATTATTTTCTATTGAAAAAACATTACAAT -22 AGATAAAAAATAAGAAGACTGC ATG ATT TAT AAA AGA GAG TTT TTC CA CACA M I R R R F F H T 25 ATG GGA AGG CAG GAT CGT ATT TAT AAA ACG CTT TCA GAA GCC TTG M G R Q D R I Y K T L S E A L 70 AAA ACA GAC AAG ATT ACC TTG TAT AAT GAT AGT TAT AAA CAT AGC K T D K I T L Y N D S Y K H S 115 CAT CAT ATT GCG ATG AAA GGC GTA CCA GAC ACA AAT GAG ACT CAT H H I A M K G V P D T N E T H 160 TTC CGT TTA GAA ATT GTA TCA CCA GAG TTC TCC GGG ATG TCA AGA F R L E I V S P E F S G M S R 205 GTC GCC CGA CAT CGA CTT GTT TAT GGA TTA CTA AAA GAT GAA TTT V A R H R L V Y G L L K D E F 250 GAT GGC GGC CTT CAT GCT CTC CAA ATA ACT TCT AGC AAG ACT CCT D G G L H A L Q I T S S K T P 295 GAT GAA GTT TCG TAA TTTTCGAAAAGTATTCACAGAATTACATGAAAGTATTCAGGTATATTATTA D E V S - 339 CATTTTACATTAGACAATTACATGACAGATTGAATGTGCTTGCT			g	earbo)X											
 -142 TGATGCTAAAATTTAATAATTTTACTTTAATTTCGTTAATTTTTTTT	-202	CTCTA	ACAC	TCG	rgga/	AAAG A	ATTO	CAA/	ATTO	Gaga/	4ATT(רסדדב	пп	TTGC	TAGC	AAT
 -82 TCTAAGCTGAGCGTCATTTCGGTGAACTTTTATTATTTTCTATTGATAAAAAAACATTACAAT -22 AGATAAAAAAATAAGAAGACTGC ATG ATT AGA AGA TTT TTT CAC ACA M I R R F F H T 25 ATG GGA AGG CAG GAT CGT ATT TAT AAA ACG CTT TCA GAA GCC TTG M G R Q D R I Y K T L S E A L 70 AAA ACA GAC AAG ATT ACC TTG TAT AAT GAT AGT TAT AAA CAT AGC K T D K I T L Y N D S Y K H S 115 CAT CAT ATT GCG ATG AAA GGC GTA CCA GAC ACA AAT GAG ACT CAT H H I A M K G V P D T N E T H 160 TTC CGT TTA GAA ATT GTA TCA CCA GAG TTC TCC GGG ATG TCA AGA F R L E I V S P E F S G M S R 205 GTC GCC CGA CAT CGA CTT GTT TAT GGA TTA ACT AGC AAG GAC TACT 206 GG C CTT CAT GCT CTC CAA ATA ACT TCT AGC AAG ACT CCT D G G L H A L Q I T S S K T P 295 GAT GAA GTT TCG TAA 295 GAT GAA GTT TCG TAA 296 CATTTTTACATTTAGACAATTACATGACAGATTGCAATGTGCTTGCT	-142	TGATO	CTA		FTAA 1	ΓΑΑΤΙ	TTAC		ATT	rcgt	ΓΑΑΤΠ	ПП	ICTCA	ACTA	AGAA	ATT
-22 AGATAAAAAATAAGAAGACTGC ATG ATT AGA AGA TTT TTT CAC ACA M I R R F F H T 25 ATG GGA AGG CAG GAT CGT ATT TAT AAA ACG CTT TCA GAA GCC TTG M G R Q D R I Y K T L S E A L 70 AAA ACA GAC AAG ATT ACC TTG TAT AAT GAT AGT TAT AAA CAT AGC K T D K I T L Y N D S Y K H S 115 CAT CAT ATT GCG ATG AAA GGC GTA CCA GAC ACA AAT GAG ACT CAT H H I A M K G V P D T N E T H 160 TTC CGT TTA GAA ATT GTA TCA CCA GAG TTC TCC GGG ATG TCA AGA F R L E I V S P E F S G M S R 205 GTC GCC CGA CAT CGA CTT GTT TAT GGA TTA CTA AAA GAT GAA TTT V A R H R R L V Y G L L K D E F 250 GAT GGC GGC CTT CAT GCT CTC CAA ATA ACT TCT AGC AAG ACT CCT D G G L H A L Q I T S S K T P 295 GAT GAA GTT TCG TAA TTTCGAAAAGTATTCACTGACAGATTGAATGTGCTTGCTCGATTGTTTTGTTG 399 CACTTTGTCGCATGCTTAAATATGAAATACTGCAAATGTGAAAAGAATTCACAGCAATTT 459 ACAAAAAGGGTGAAACCAAATTACATGACAGATTGAATGTGATTGACTGAAAAGCAATAACCAAAA 639 ATATTTACTTAAATTAGGAGTATTTAAAGAGATGTTACAAGAAACCAAAAACGAAAAG 640 TTTACTTAAATTAGGAGTATTTAAAGAGATGTTACAAGAAAACCAAAAACAGAAAA 579 AGCACTTCAATGGAGTATTTAAAGAGATTTAACTAAGAAAACCAAAAAACCAAAAACAGAAAA 579 AGCACTTCAGCTGATAGGAGTATTTAAAGAGATTTAACCATAAGGGTCATCAAAGC 699 AGAAACTGGGTAAACTTACGTTGCTTAAAATACTTAACTTAAAGAAATCCATAAGGAAATCGTTAAGCAAATGGTATACTAAGCAATTAACTTAACTAAGAAATCCATAAGGAAATCCATAAAGCAAAACCAAAAAGC 699 AGAAACTGGGTAAACTTACATGGCAGATTTAACTAAAACCAAAAACCAAAAGC 699 AGAAACTGGGTAAACTGGTTAAAATTCGTTTAGCAAGTTTAACCATAAGAAATCCATAAGCT 690 AGAAACTGGGTAAACTGGTTAAAATTCGTTTACCAAGGATTTAACTAAGGAATCGTTAACCATAAGCAAAACCAAAACCAAAACCAAAACCAAAACCAAAACCAAAA	-82	TCTAA	GCTO	CAGCO	STCAT	птс	GTG/	ACTI	TTAT	TAT	птст	TATTO	GAAAA	AACA	TTAC	AAT
$ \begin{array}{c} M I R R F F H T \\ 125 ATG GGA AGG CAG GAT CGT ATT TAT AAA ACG CTT TCA GAA GCC TTG \\ M G R Q D R I Y K T L S E A L \\ 170 AAA ACA GAC AAG ATT ACC TTG TAT AAT GAT AGT TAT AAA CAT AGC \\ K T D K I T L Y N D S Y K H S \\ 115 CAT CAT ATT GCG ATG AAA GGC GTA CCA GAC ACA AAT GAG ACT CAT H H I A M K G V P D T N E T H \\ 160 TTC CGT TTA GAA ATT GTA TCA CCA GAG TTC TCC GGG ATG TCA AGA F R L E I V S P E F S G M S R \\ 205 GTC GCC CGA CAT CGA CTT CTT TAT GGA TTA CTA AAA GAT GAA TTT V A R H R L V Y G L L K D E F \\ 250 GAT GGC GGC CTT CAT GCT CTC CAA ATA ACT TCT AGC AAG ACT CCT D G G L H A L Q I T S S K T P \\ 295 GAT GAA GTT TCG TAA TGAA TTTTCGAAAAGTATTCAGGGTATATTATTA D E V S - \\ 339 CATTTTTACATTTACATTACATTACATGACAATTACATGACAGATTGAATGTGCTTGCT$	-22	AGATA			AGAAC	БАСТО	C.		ATG	ATT	AGA	AGA	TTT	TTT	CAC	ACA
25 ATG GGA AGG CAG GAT CGT ATT TAT AAA AGG CTT TCA GAA GCC TTG M G R Q D R I Y K T L S E A L 70 AAA ACA GAC AAG ATT ACC TTG TAT AAT GAT AGT TAT AAA CAT AGC K T D K I T L Y N D S Y K H S 115 CAT CAT ATT GCG ATG AAA GGC GTA CCA GAC ACA AAT GAG ACT CAT H H I A A M K G V P D T N E T H 160 TTC CGT TTA GAA ATT GTA TCA CCA GAG TTC TCC GGG ATG TCA AGA F R L E I V S P E F S G M S R 205 GTC GCC CGA CAT CGA CTT GTT TAT GGA TTA CTA AAA GAT GAA ATT V A R H R L V Y G L L K D E F 250 GAT GGC GGC CTT CAT GCT CTC CAA ATA ACT TCT AGC AAG ACT CCT D G G L H A L Q I T S S K T P 295 GAT GAA GTT TCG TAA TTTTCGAAAAGTATTCAGCAAGATTGAATGTGCTTGCTCGATTGTTTGT									M	т	R	R	F	F	Н	Т
M G R Q D R I Y K T L S E A L 70 AAA ACA GAC AAG ATT ACC TTG TAT AAT GAT AGT TAT AAA CAT AGC K T D K I T L Y N D S Y K H S 115 CAT CAT ATT GCG ATG AAA GGC GTA CCA GAC ACA AAT GAG ACT CAT H H I A M K G V P D T N E T H 160 TTC CGT TTA GAA ATT GTA TCA CCA GAG TTC TCC GGG ATG TCA AGA F R L E I V S P E F S G M S R 205 GTC GCC CGA CAT CGA CTT GTT TAT GGA TTA CTA AAA GAT GAA TTT V A R H R L V Y G L L K D E F 250 GAT GGC GGC CTT CAT GCT CTC CAA ATA ACT TCT AGC AAG ACT CCT D G G L H A L Q I T S S K T P 295 GAT GAA GTT TCG TAA TTGAATAGCAATTGAATGTGCTTGCTCGATTGTTTGTTG 399 CACTTTGTTCGAAACAATTACATGACAATTACATGAAAGAATTTCAACGAAAACAAAAACAAAA 59 ACAAAAAGGGTGAAACCAAAATAAATCTTATTTATTTA CTTAAAAAGGGTAAAACAAAAC	25	ATC	CCA	ACC	CAG	САТ	ССТ	ATT	TAT	<u>۸۸۸</u>	ACC	cπ	TCA		CCC	ттс.
70 AAA ACA GAC AAG ATT ACC TTG TAT AAT GAT AGT TAT AAA CAT AGC K T D K I T L Y N D S Y K H S 115 CAT CAT ATT GCG ATG AAA GCC GTA CCA GAC ACA AAT GAG ACT CAT H H I A M K G V P D T N E T H 160 TTC CGT TTA GAA ATT GTA TCA CCA GAG TTC TCC GGG ATG TCA AGA F R L E I V S P E F S G M S R 205 GTC GCC CGA CAT CGA CTT GTT TAT GGA TTA CTA AAA GAT GAA TTT V A R H R L V Y G L L K D E F 250 GAT GGC GGC CTT CAT GCT CTC CAA ATA ACT TCT AGC AAG ACT CCT D G G L H A L Q I T S S K T P 295 GAT GAA GTT TCG TAA TTTTCGAAAAGTATTCAGCAATTACATGAAAGGATTGAATGTGCTTGCT	23		000	DDA		n	0	T	^v	- NAA	т	-	c	E	Å	1
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k i b k i i i c y n b b s y k h s 115 CAT CAT ATT GCG ATG AAA GGC GTA CCA GAC ACA AAT GAG ACT CAT h h i a m k g v p b t n e t h 160 TTC CGT TTA GAA ATT GTA TCA CCA GAG TTC TCC GGG ATG TCA AGA F R L e i v s p e f s g m s r 205 GTC GCC CGA CAT CGA CTT GTT TAT GGA TTA CTA AAA GAT GAA TTT v A R h R L v y g L L k d e f 250 GAT GGC GGC CTT CAT GCT CTC CAA ATA ACT TCT AGC AAG ACT CCT b g g L h A L Q i t s s k t p 295 GAT GAA GTT TCG TAA TTTTCGAAAAGTATTCAGCAGATTGAAAGTATTCAGGTATATTATTA b e v s - 339 CATTTTTACATTTAGACAATTACATGACAGATTGAATGTGCTTGCT	70	AAA	ACA	UAC	AAG	ALL	ACC	110			GAT	AUI		AAA		AUC
115 CAT CAT ATT GCG ATG AAA GCC GTA CCA GAC ACA AAT GAG ACT CAT H H I A M K G V P D T N E T H 160 TTC CGT TTA GAA ATT GTA TCA CCA GAG TTC TCC GGG ATG TCA AGA F R L E I V S P E F S G M S R 205 GTC GCC CGA CAT CGA CTT GTT TAT GGA TTA CTA AAA GAT GAA TTT V A R H R L V Y G L L K D E F 250 GAT GGC GGC CTT CAT GCT CTC CAA ATA ACT TCT AGC AAG ACT CCT D G G L H A L Q I T S S K T P 295 GAT GAA GTT TCG TAA TTTCGAATACTGCGAAGTTGAATGGGTATATTATTA D E V S - 339 CATTTTTACATTTAGACAATTACATGACAGATTGAATGTGCTTGCT		K		U	K	1		L	Ť.	N	U		, r	K	H	2
H H I A M K G V P D T N E T H 160 TTC CGT TTA GAA ATT GTA TCA CCA GAG TTC TCC GGG ATG TCA AGA F R L E I V S P E F S G M S R 205 GTC GCC CGA CAT CGA CTT GTT TAT GGA TTA CTA AAA GAT GAA TTT V A R H R L V Y G L L K D E F 250 GAT GGC GGC CTT CAT GCT CTC CAA ATA ACT TCT AGC AAG ACT CCT D G G L H A L Q I T S S K T P 295 GAT GAA GTT TCG TAA TTTCGAAAAGTATTCAGGTATATTATTA D E V S - 339 CATTTTTACATTTAGACAATTACATGACAGATTGAATGTGCTTGCT	115	CAT	CAT	ATT	GCG	ATG	AAA	GGC	GTA	CCA	GAC	ACA	AAT	GAG	ACT	CAT
160 TTC CGT TTA GAA ATT GTA TCA CCA GAG TTC TCC GGG ATG TCA AGA F R L E I V S P E F S G M S R 205 GTC GCC CGA CAT CGA CTT GTT TAT GGA TTA CTA AAA GAT GAA TTT V A R H R L V Y G L L K D E F 250 GAT GGC GGC CTT CAT GCT CTC CAA ATA ACT TCT AGC AAG ACT CCT D G G L H A L Q I T S S K T P 295 GAT GAA GTT TCG TAA D E V S - 339 CATTITTACATTTAGACAATTACATGACAGATTGAATGTGCTTGCT		Н	Н	Ι	Α	м	ĸ	G	v	Р	D	Т	Ν	Ε	Т	Н
FRLEIVSPEFSGMSR205GTCGCCCGACATCGACTTGTTTATGGATTACTAAAAGATGAATTTVARHRLVYGLLKDEF250GATGGCGGCCTTCATGCTCTCCAAATAACTTCTAGCAAGAGTCCTDGGLHALQITSSKTP295GATGAAGTTTCGTAATTTTCGAAAAGGTATTCAGGTATATTATTADEVS-339CATTTTTACATTACATTAGACAATTACATGACAGATTGAATGTGGCTTGCTCGATTGTTTGT	160	ттс	CGT	TTA	GAA	ATT	GTA	TCA	CCA	GAG	ΤТС	TCC	GGG	ATG	TCA	AGA
205 GTC GCC CGA CAT CGA CTT GTT TAT GGA TTA CTA AAA GAT GAA TTT V A R H R L V Y G L L K D E F 250 GAT GGC GGC CTT CAT GCT CTC CAA ATA ACT TCT AGC AAG ACT CCT D G G L H A L Q I T S S K T P 295 GAT GAA GTT TCG TAA TTTTCG TAA TTTTCGAAAAGTATTCAGGTATATTATTA D E V S - 339 CATTTTTACATTACATTAGACAATTACATGAACAGATGAAAGGATTCAGGATGTTTGTT		F	R	L	Ε	I	V	S	Ρ	Е	F	S	G	м	S	R
V A R H R L V Y G L L K D E F 250 GAT GGC GGC CTT CAT GCT CTC CAA ATA ACT TCT AGC AAG ACT CCT D G G L H A L Q I T S S K T P 295 GAT GAA GTT TCG TAA TTTTCGAAAGTATTCAGGTATATTATTA D E V S - 339 CATTTTTTTTTTTTTTTTTAGACAATTACATGACAGATTGAATGTGCTTGCT	205	GTC	372	CGA	CAT	CGA	СТТ	GTT	TAT	GGA	TTA	СТА	AAA	GAT	GAA	Π
250 GAT GGC GGC CTT CAT GCT CTC CAA ATA ACT TCT AGC AAG ACT CCT D G G L H A L Q I T S S K T P 295 GAT GAA GTT TCG TAA TTTTCGAAAAGTATTCAGGTATATTATTA D E V S $-$ 339 CATTTTTACATTTAGACAATTACATGACAGATTGAATGTGCTTGCT		v	Δ	R	н	R	1	v	Y	G	1	1	K	D	F	F
 D G G L H A L Q I T S S K T P 295 GAT GAA GTT TCG TAA TTTTCGAAAAGTATTCAGGTAATTATTA D E V S - 339 CATTTTTACATTTAGACAATTACATGACAGATTGAATGTGCTTGCT	250	CAT	ccc	ccc	ĊTT	CAT	сст	cic	CAA	ΔΤΔ	ACT	TCT	ACC		ACT	сст ССТ
 295 GAT GAA GTT TCG TAA 295 GAT GAA GTT TCG TAA 295 TTTTTACATTTCG TAA 339 CATTTTTACATTTAGACAATTACATGACAGATTGAATGTGCTTGCT	200			C					0	T	<u>т</u>	c	C C	NAG K	T	
 295 GAT GAA GITTICG TAA D E V S - 339 CATTITTACATTTAGACAATTACATGACAGTGAAAGTATTGAGTGGTTGCTCGGATGTTTTGTTG 399 CACTTTGTTCGCATGCTTAAATATGAAATACTGCGAAGTGAAAAGAATTTCACACATATT 459 ACAAAAAGGGTGAAACCAAAATAAAATCTTATTTTGAGTTGAGTTGATTCACGGCTTAATCAGCGA poly(A)-addition signal 519 TTATTTACTTAAATTAGTATACACGGTGATTGCCAATATGTACCGTCGATTCTACAAAACAAAACAAAAACAAAAACAAAAACAAAAACAAAA	205	CAT	C	CTT U	TCC	П	A	Ľ	ч _т		~~~~			СТАТ		г ттл
D E V S - 339 CATTITTACATTTAGACAATTACATGACAGATTGAATGTGCTTGCT	295	GAI	GAA	GIL	icu	IAA			I	Πü	JAAAA	ACTA:	ITCAC	GIAI	ALIA	IIA
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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 (*MluI* 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 $1 \times$ 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 DNAbinding 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/m² 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 *EspI-RsaI* 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/m², 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 MluI cell cycle box (MCB) elements, TCGTGT and AGGCGT, present with one and two mismatches to S. cerevisiae MCB element (ACG-CGT) at nt positions -493 and -504, respectively. Interestingly, four putative SWI4/6-dependent cell cycle box (SCB) elements, GACAAAAA, AAAGAAAA, CTC-AAAAA, 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) (AGG-

AAAGAAA) 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) tempera-

ture. 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/m² 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 ftsZdependent 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. Furthermore, 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.

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