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Identification of a Regulatory Element Required for 3′-End Formation in Transcripts of *rhp51*$^+$, a *recA* Homolog of the Fission Yeast *Schizosaccharomyces pombe*

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Our previous report demonstrated that the *rhp51*$^+$, a *recA* and *RAD51* homolog of the fission yeast, encodes three transcripts of 1.9, 1.6 and 1.3 kb which have at least six polyadenylation sites. The 3′-end of the gene alone can direct the formation of multiple, discrete 3′ ends of the transcripts. To identify the regulatory element required for the 3′-end formation of *rhp51*$^+$, deletion mapping analysis was performed. Northern blot analysis revealed that the 254-bp DNA fragment including 4 distinct poly (A) sites downstream from the *Hin*dIII site, is crucial for normal 3′-end formation. Deletion of the 3′-terminal AU rich region caused appearance of read-through RNA, leading to enhancement of survival rate of the *rhp51* deletion mutant in response to DNA damaging agent, methylmethane sulfonate (MMS). The results imply that the *rhp51*$^+$ system may be useful for molecular analysis of the 3′-end formation of RNA in the fission yeast.

While clear evidence for the existence of common regulatory mechanisms controlling the induced transcription of genes in response to DNA damage is lacked in eukaryotic systems such as yeast, several reports have indicated the existence of sensory responses to DNA damage in *Saccharomyces cerevisiae* (Friedberg et al., 1995). Regulation of gene expression occurs at both transcriptional and post-transcriptional levels. Most of works were largely accomplished on transcription initiation controlled by transcription factors and their *cis*-acting sequence on the promoter. However, there are little known about post-transcriptional regulation including splicing, 3′-end formation of mRNA and poly (A) addition. Recently, several lines of evidence supported that efficient 3′-end formation of mRNA plays a crucial role in gene regulation (Guo and Sherman, 1996).

We have previously shown that the mRNA levels of *rhp51*$^+$, a *recA* and *RAD51* homolog from the fission yeast *Schizosaccharomyces pombe*, increase in response to DNA damaging agents. Three transcripts of *rhp51*$^+$ were differentially regulated during the mitotic cell cycle and the growth cycle. Interestingly, the *rhp51*$^+$ gene has at least 6 polyadenylation sites (Jang et al., 1996). While damage-response of *rhp51*$^+$ was controlled by its promoter region, the 3′-terminal region was involved in the formation of multiple and discrete 3′ ends of the transcripts. Based on knowledge, in this work it is shown that the 3′-terminal region including 254-bp DNA fragment downstream from the *Hin*dIII, is important for 3′-end formation of *rhp51*$^+$.

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Materials and Methods

The *S. pombe* strains used were JY334 (*h*$^+$ *ade6-M216 leu1-32*) and JAC1/51 (*h*$^+$ *ade6-704 leu1-32 ura4-D18 rhp51::ura4*$^+$) obtained from Drs. M. Yamamoto and A. M. Carr, respectively. pWH5 and Splac551 vectors have been previously described (Jang et al., 1996; our unpublished data). Growth media for *S. pombe* and its transformation protocol were described (Moreno et al., 1991).

Isolation of total RNA and Northern blot analysis were carried out as described by Jang et al. (1996).

For survival test of methylmethane sulfonate(MMS), transformants were grown on selective medium overnight and plated onto minimal medium containing appropriate MMS concentrations and then the number of the surviving colonies were counted after 4-5 days of incubation.

Results and Discussion

Deletion mapping of 3′-terminal region of rhp51$^+$ *for analysis of 3′-end formation of the mRNA*

To identify the regulatory elements required for 3′-end

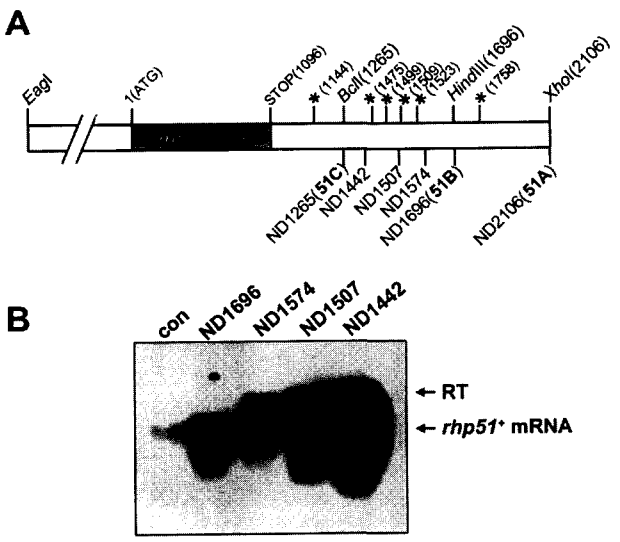

Fig. 1. Deletion mapping of 3′ terminal regions of the *rhp51*⁺ gene. A, Restriction map of the 4.6 kb *Eag*I-*Xho*I fragment containing *rhp51*⁺. The nt positions are shown with the first nt of the first Met codon of the Rhp51 protein designated as +1 (Jang et al., 1996). Asterisks indicate the six polyadenylation sites. B, Northern blot analysis for detection of mRNA derived from each deletion construct based on a plasmid Splac551. Wild type cells were transformed with each construct indicated. Total RNA was isolated and used for Northern blot using a 0.4 kb *Eco*RI fragment of *rhp51*⁺ as a probe. RT, readthrough RNA.

formation of *rhp51*⁺ mRNA, deletion constructs for the 3′-terminal region of the gene were made (Fig. 1A). Each construct was used for transformation of wild type or *rhp51* mutant cells. The Leu⁺ transformants were isolated and cultured in selective medium overnight. After collection of the cells, total RNAs were extracted and subjected to Northern blot analysis for detection of *rhp51*⁺ mRNA using a 0.4 kb *Eco*RI fragment of *rhp51*⁺ as a probe. The data demonstrated that a readthrough RNA appeared first from ND1574 and strongly accumulated in ND1442 (Fig. 1B). The results suggest that the 254-bp DNA fragment between ND1696 and ND1442 is required for faithful 3′-end formation. Recent reports demonstrated that the 3′-end AU rich sequence of the *S. pombe ura4*⁺ gene is required for 3′-end formation and acts as a site determining signal (Birse et al., 1997; Humphrey et al., 1994). But there was no significant sequence similarity between 3′-end sequences of *ura4*⁺ and *rhp51*⁺ except for AU richness (Hunphrey et al., 1994; Jang et al., 1996). These data imply that action mechanisms of the 3′-end formation of yeast mRNA may be dependent in a gene-specific manner.

Accumulated RNA which was caused by deletion of 3′ -end-forming signals contributes to enhancement of MMS survival rate in rhp51 mutant

To examine whether the increased RNA level of *rhp51*⁺ induced by deletion of the 3′-end sequence responds to treatment of DNA damaging agents and affects cell survival, Northern blot hybridization analysis and cell

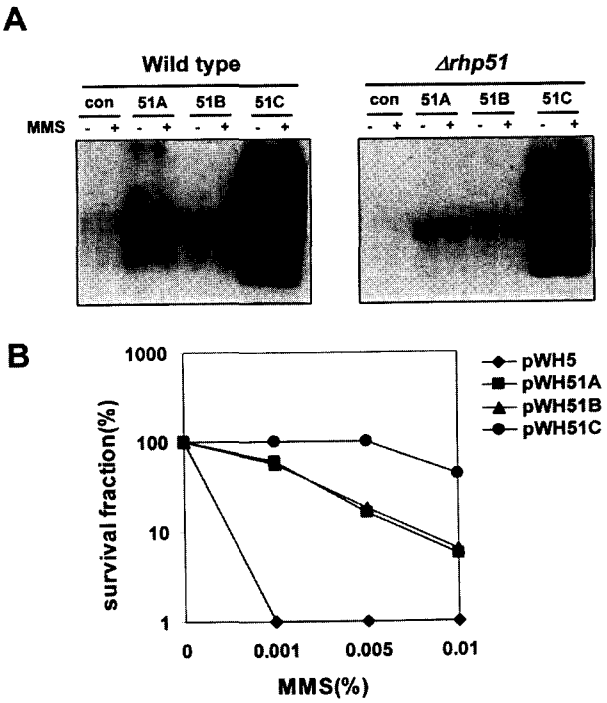

Fig. 2. Deletion of the C-terminal region including multiple poly A sites increases the mRNA level and leads to cause *rhp51* deletion mutant cells to become more resistant to MMS treatment. A, Northern blot analysis using total RNA extracted from yeast transformants by each construct (for more detail information, see Fig. 1A). 51A, 51B and 51C were constructed by subcloning the designated DNA fragments into pWH5 vectors, respectively. B, Effect of increased RNA levels on MMS survival of the *rhp51* deletion mutant transformed with each construct as describe above.

survival test were performed. Fig. 2A showed that the accumulated RNA of *rhp51*⁺ by deletion of the 3′-end DNA fragment including 4 poly (A) sites was not induced by MMS treatment. However, the increased level of RNA including read-through RNA caused enhanced survival of *rhp51* mutant cells to MMS (Fig. 2B). The data were a little surprising because overexpression of *rhp51*⁺ caused severe growth defect (our unpublished data). Recent report demonstrated that 2-3 fold overexpression of hamster Rad51 increased resistance of mammalian cells to ionizing radiation (Vispe et al., 1998). Therefore, it is possible to speculate that the increased levels of mRNA by deletion of 3′-end-forming signals in *rhp51*⁺ resulted in a moderate overexpression of Rhp51 protein, leading to help *rhp51* mutant cells to be radiation-resistant. In conclusion, these data suggest that the mRNA level of *rhp51*⁺ might be differentially controlled by selective polyadenylation during the mitotic cell cycle and growth cycle.

The nature of 254-bp sequence including 4 poly (A) sites remains to be examined in more detail. In addition, it will be much interesting to identify the *trans*-acting factor which binds to the sequence. This work provides a useful model system to elucidate the 3′-end formation mechanism of eukaryotic mRNA as well as the induction mechanism in response to DNA

damage from the fission yeast *S. pombe*.

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