Environmental Mutagens & Carcinogens 22-3 : 137-141 (2002)

Characterization of *hrp2+* Gene Related to SNF2 Family in *Schizosaccharomyces pombe*

In Soon Choi
Department of Life Sciences, Silla University, Pusan 617-736

(Received August 2, 2002 / Accepted September 10, 2002)

ABSTRACT : **The SNF2/SW12 family comprises proteins from a variety of species with *in vivo* functions, such as transcriptional regulation, maintenance of chromosome stability during mitosis, and various types of DNA repair. This study was shown the characterization of *hrp2+* gene which was isolated by PCR amplification using the conserved domain of SNF2 motifs. Sequence analysis of *hrp2+* gene showed striking evolutionary conservation among the SNF2 family of proteins. The transcript of *hrp2+* gene was found to be a 4.7 kb as identified by Northern hybridization. In addition, to determine the transcription initiation site of *hrp2+* gene, primer extension analysis was performed. This result showed the band of 64 bp. The transcriptional start point was mapped to a position of 47 base pair from the first ATG codon of translational initiation codon. In order to investigate the inducibility of *hrp2+* gene, transcript levels were examined after treating the cells to various DNA damaging agents. The transcripts of *hrp2+* were induced by UV-irradiation. But the transcripts were not induced by treatment of 0.25% Methylmethane sulfonate (MMS). These results implied that the effects of damaging agents are complex and different regulatory pathways exist for the induction of this gene.**

Keywords: SNF2/SW12 family, *hrp2+* gene, primer extension, UV-induction

Introduction

The yeast SWI2/SNF2 gene was identified in genetic spectrum of differently regulated yeast genes (Peterson *et al.*, 1994). Transcriptional dependence on SWI2/SNF2 appears to be related to the nature of the core promoter sequence, the number of binding sites for upstream activators, and the type of activator (Laurent *et al.*, 1993). The SWI2/SNF2 polypeptide contains the characteristic seven conserved protein motifs that are present in a large and rapidly growing group of nucleoside triphosphate (NTP)-binding proteins that include DNA and RNA helicases (Gorbalenya and Koonin 1993; Carlson and Laurent, 1994; Eisen *et al.*, 1995).

The number of proteins assigned to the SNF2 family has increased rapidly over the last few years and continues to expand. Interestingly, members of the SNF2-like family exhibit an impressive range of biological functions. The activities include gene-specific transcriptional activation (SNF2 subfamily), transcriptional repression (MOT1), destabilization of reconstituted nucleosomes (SNF2 and SNF2L subfamilies), transcriptioncoupled repair (ERCC6 subfamily), nucleotied excision repair of nontranscribed region of the genome (RAD16), recombination repair (RAD54 subfamily), chromosome segregation (lodestar) and chromatin compaction (CHD1 subfamily). Some of the proteins in the SNF2 family contain sequence motifs, which help identify the function conserved within the subfamily. All members of the SNF2 subfamily contain a chromodomain motif. This motif is found in a variety of proteins involved in transcriptional regulation (Cairns *et al.*, 1994) and it has been suggested that it may be involved in protein-protein interactions. CHD1 subfamily contains a chromodomain motif, which is found in a few other proteins and is proposed to play a role in chromatin compaction (Bork and Koonin, 1993). Finally, a RING finger motif is found in all the proteins in the RAD16 subfamily. This motif is related at the sequence and structural levels to the zinc finger motif (Lovering *et al.*, 1993; Bauer *et al.*, 1994). It is found in many proteins that interact with DNA (including the DNA repair protein RAD18, the p53-associated protein MDM2 and the protooncogene mel-18) and suggested that it is involved in DNA binding (Lovering *et al.*, 1993).

Recently, the number of proteins in this family is

*To whom all correspondence should be addressed

increasing rapidly through genome sequencing projects or homology-based cloning. This study was aimed to isolate unidentified SNF2/SWI2 family proteins and to investigate the conservation of sequence and function within eukaryotes. The fission yeast *Schizosaccharomyces pombe* was used for this study, which serves as an important model system for the study of basic processes in higher eukaryotes.

Materials and Methods

Strains, cell culture, and genetic methods

E. coli strain DH5α (F- endA1, hsd17, (r-, mk-), supE44, thi-1, recA1, gyrA96, relA1, lacIqZ-M15) and yeast *S. pombe* strain JY741 (h- ade6- M210 leu1-32 ura4-D18) was used for this study. *E. coli* strains were grown on LB media (1% tryptone, 1% sodium chloride, 0.5% yeast extract). Yeast strain was grown in YE (2% glucose, 0.5% yeast extract) medium supplemented with appropriate amino acids. Standard molecular biology techniques were employed as described (Sambrook and Russell, 2001). *S. pombe* chromosomal DNAs were prepared according to the methods of Cryer *et al.*, (1975).

Isolation of RNA and Northern blot analysis

Total RNA was prepared according to Jang *et al.* (1995). RNA was denatured, fractionated on 1.2% agarose gel containing 0.66 M formaldehyde, and transferred onto S&S Nytran membrane. The probe and filter hybridization and washing conditions were identical to those of Southern blot analysis.

Preparation of DNA probe by random priming

The DNA fragment was labeled with [α-^{32}P] dCTP (3,000 Ci/mmole) by random primed DNA labeling method (Feinberg and Vogelstein, 1984). The labeling reaction was carried out in 20 ul of the standard random priming buffer containing 50 ng of DNA, 30 uCi of [α-^{32}P] dCTP, dATP, dGTP, dTTP and 2 unit of Kenow enzyme for 1 hr at 37°C.

UV-light and MMS treatment

One hundred milliliters of cells grown to mid exponential stage were harvested, washed, and then resuspended in 10 ml of distilled water. The cell suspension was evenly spread onto 150 mm-petridish and exposed to 200 J/m^2 of ultraviolet (UV)-light or 0.25% MMS treatment. The treated cells were inoculated into fresh YES medium, incubated at 30°C in the dark, and collected at indicated times.

Primer extension and Nucleotide sequencing

A 20-mer oligonucleotide corresponding to the region around the first in frame ATG of *hrp2+* gene was synthesize and used as primer. The primer labeling and the extension by reverse transcriptase were done as described by Sambrook and Russell (2001). An oligonucleotide was 5'-labeled with polynucleotide kinase and [γ-^{32}P] ATP and used to hybridized with 50 μg of total RNA extracted from *S. pombe* cells. Hybridization was performed at 30°C for 16 h and then extension by avian myeloblastosis virus reverse transcriptase was done at 42°C for 2 h. The extended products were fractionated on an 8 M urea/6% polyacrylamide gel along with Sanger dideoxy sequencing reactions as standards.

Plasmid DNA that was to be sequenced was purified by a plasmid preparation kit (Qiagen). An ABI PRISM 377 DNA sequencer (Perkin-Elmer) analyzed the nucleotide sequence. The nucleotide sequence and inferred amino acid sequences were aligned by GenBank database.

Results and Discussion

Isolation and nucleotide sequence of *hrp2+* gene from *S. pombe*

To isolate new members of SNF2 helicase related genes from *S. pombe*, this study took advantage of the highly conserved sequence elements within the *ERCC6* gene and generated the degenerative oligo primers (Troelstra *et al.*, 1992; Yoshinaga *et al.*, 1992; Elfring *et al.*, 1994; Kornberg and Lorch, 1995; Jin *et al.*, 1996). Sequence homologies in domain I and III allowed the design of PCR primers (Choi, 1999; Kang and Choi, 2000). This work was focused on hrp2 PCR product containing 438 bp DNA. The nucleotide sequence of hrp2 PCR product was determined (Jin *et al.*, 1996) and amino acid sequences of the amplified regions of hrp1 and hrp2 were aligned with the corresponding regions of ERCC6 and MOT1 proteins using Multalin software. Examination of the alignment revealed that the four regions of amino acid identity were clustered.

Based on these results, hrp2 PCR product can be used as a DNA probe for isolation of helicase related genes. The helicase related gene (designated as *hrp2+*) in *S. pombe* was isolated by screening genomic libraries constructed in pIRT5. The screening of library was

initiated with the 438 bp PCR product as a probe. Among 40,000 colonies screened, a clone with 6.5 kb insert DNA was isolated, and then sequenced (Choi, 1999; 2001). From these nucleotide sequence, amino acid sequence were analyzed. This result showed that Hrp2 has a chromodomain, ATPase/7helicase domain, and one DNA binding domain (Fig. 1).

Identification of *hrp2+* transcripts

In order to identify the transctipt size of *hrp2+* gene, northern analysis was performed. Total yeast RNA from *S. pombe* was hybridized with the denatured cloned DNA. The size of transcript was found to be a 4.7 kb as identified by northern blot (Fig. 2). To determine the transcription initiation site of the *hrp2+* gene start, primer extension analysis was employed using the 20-mer oligonucleotide. This result showed a extended band of 64 bp. The transcriptional start point was mapped to a position of 47 base pair from the first ATG codon of translational initiation codon (Fig. 3).

To examine the transcriptional regulation of *hrp2+* gene by treatment of DNA damagng agents, total RNA from the cells was treated with UV and MMS. The UV-irradiation (200 J/m^2) increased *hrp2+* gene expression in time dependent manners (Fig. 4). But MMS (0.25%) treatment did not increase (Fig. 4). These results implied that the effects of damaging agents are complex and different regulatory pathways exist for the induction of this gene. Also, the expression of the gene increased after UV-irradiation, indicating that the *hrp2+* is a novel UV-inducible gene in *S. pombe*. To determine the level of *hrp2+* gene during cellular growth, JY741 cells were cultured in rich medium and collected at every one or two

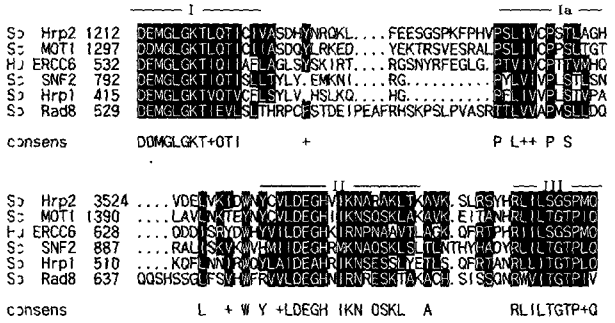

Fig. 1. Comparison of Hrp2 with CHD1 subfamily members. Schematic representation of Hrp2, SYGP-4, mCHD1 and dCHD1 to indicate the location of the homologous segment in the proteins. Chromodomain (C) is lightly shaded: ATPase/7 helicase domain is indicated by the closed box; the region of DNA binding domain (D) is darkly shaded. The numbers at the ends of diagrams denote the length of each polypeptide. Schematic drawing of the *hrp2*$^+$ gene shows that the protein product encodes 1548 amino acids.

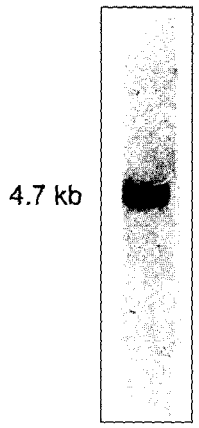

Fig. 2. Northern blot analysis of *hrp2+* mRNA in *S. pombe*. Total RNA was isolated, electrophoresed, transferred onto nitrocellulose filter, and then hybridized with the radiolabelled *hrp2+* DNA probe. The size of transcript is 4.7 kb.

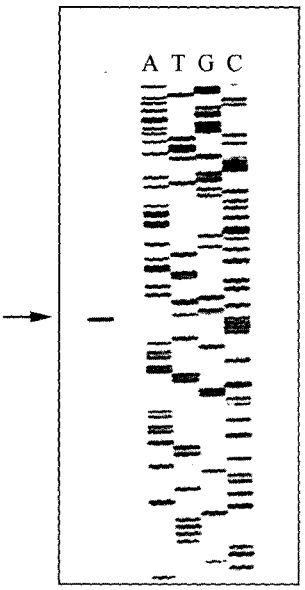

Fig. 3. Primer extension analysis of *hrp2+* gene. To determine the transcription initiation site of *hrp2+* gene, primer extension analysis was performed. The 20- mer oligonucleotide, 5' GAAACGGGCATCCACGCGAC 3' from −145 to −164 was synthesized and used as primer. About 5×10^5 cpm of ^{32}P-labelled primer was hybridized with 100 μg of total RNA from *S. pombe* cells. After extension reaction using AMV reverse transcriptase, the size of synthesized cDNA was analyzed on 8% polyacrylamide gel containing 8 M urea. Lane A, T, G, C; nucleotide sequence of M13mp18 as size marker.

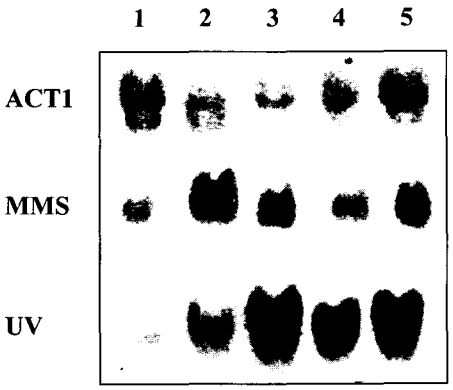

Fig. 4. The transcript levels of *hrp2+* gene in UV and MMS treated cells. Cells in log phase were treated with 200 J/m^2 UV and 0.25% MMS, and then incubated at 30°C. After incubating, RNA was isolation and northern blot analysis was performed. The numbers at the top of each lane indicate the cell-collection times (in hours) after the treatment. ACT1 was used as an internal control.

hours to prepare total RNA. The Northern blot analysis showed that the level of *hrp2+* transcript reached its maxim before the cells entered the exponential growth phase and then decreased gradually. This suggests that *hrp2+* gene is expressed mainly at the early stage of cell growth (Choi, 2001).

References

Bauer, W.R., J.J. Hayes, J.H. White, and A.P. Wolffe, (1994): Nucleosome structural changes due to acetylation. *J. Mol. Biol.* **236**, 685-690.

Bork, P., and E.V. Koonin, (1993): An expanding family of helicases within the DEAD/H subfamily. *Nucleic Acids Res.* **21**, 751-752.

Cairns, B.R., J.-Y. Kim, M.H. Sayre, B.C. Laurent, and R.D. Kornberg, (1994): A multisubunit complex containing the SWI1/ADR6, SWI2/SNF2, SWI3/SNF5, and SNF6 gene products isolated from yeast. *Proc Natl Acad Sci. USA* **91**, 1950-1954.

Carlson, M. and B.C. Laurent, (1994): The SNF/SWI family of golbal transcriptional activaators. *Curr. Opin. Cell. Biol.* **6**, 396-402.

Choi, I.S. (1999): Isolation and characterization of new family genes DNA damage in yeast. *Environmental Mutagens & Carcinogens* **19(1)**, 28-33.

Choi, I.S. (2001): The study of transcriptional regulated gene, *hrp2+*, in yeast. *K. J. Life Science.* **11-2**, 111-115.

Cryer, D.R., R. Eccleshull, and J. Marmur, (1975): Isolation of yeast DNA. In: Methods in Cell Biology ?. Academic Press, pp39-44.

Eisen, J.A., K.S. Sweder, and P.C. Hanawalt, (1995): Evolution of the SNF2 family of proteins: subfamilies with distinct sequences with functions. *Nucleic Acids Res* **23(14)**, 2715-2723.

Elfring, L.K., R. Deuring, C.M. McCallum, C.L. Peterson, and J.W. Tamkun, (1994): Identification and characterization of *Drosophila* relatives of the yeast transcriptional activator SNF2/SWI2. *Mol. Cell. Biol.* **12**, 2225-2234.

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

Gorbalenya, A.E., and E.V. Koonin, (1933): Helicaes: amino acid sequence comparisons and stucture function relationship. *Curr. Opin. Sturct. Biol.* **3**, 419-429.

Jang, Y.K., Y.H. Jin, M.J. Kim, R.H. Seong, S.H. Hong, and S.D. Park, (1995): A simple and efficient method for the isolation of total RNA from the fission yeast. *Schizosaccharomyces pombe. Biochem. Mol. Biol. Int.* **37**, 339-344.

Jin, Y.H., Y.K. Jang, M.J. Kim, J.B. Koh, J.K. Park, I.S. Choi, and S.D. Park, (1996): Isolation of *hrp2*$^+$ gene, a new member of SNF2/SWI2 family from fission yeast. *Schizosaccharomyces pombe. Mol Cells* **6**, 504-507.

Kang, S.A., and I.S. Choi (2000): Molecular cloning and characterization of DNA repair related gene in yeast. *K. J. Life Science.* **10-1**, 40-44.

Kornberg, R.D., and Y. Lorch, (1995): Interplay between chromatin structure and transcription. *Cuur. Opin. Cell. Biol.* **7**, 371-375.

Laurent, B.C., I. Treich, and M. Carlson, (1993): The yeast SNF2/SWI2 protein has DNA-stimulated ATPase activity required for transcriptional activation. *Genes Dev.* **7**, 583-591.

Lovering, R., I.M. Hanson, K.L.B. Borden, S. Martin, N.J. O'Reilly, G.I. Evan, D. Rahman, D.J.C. Pappin, J. Trowscle, and P.S. Freemont, (1993): Identification and preliminary characterization of a protein motif related to the zinc finger. *Proc Natl Acad Sci. USA* **90**, 2112-2116.

Peterson, C.L., A. Dingwall, and M.P. Scott, (1994): Five SWI2/SNF gene products are components of a large multisubunit complex required for transcriptional enhancement. *Proc. Natl. Acad. Sci. USA* **91**, 2905-2908.

Sambrook, J., E.F. Fritsch, and T. Maniatis, (1989): Molecular cloning: a laboratory manual. *Cold Spring Habor Laboratory, Cold Spring Habor, New York.*

Sambroo, J., and D.W. Russell, (2001): Molecular cloning: a laboratory manual (3rd edition). *Cold Spring Habor Laboratory, Cold Spring Habor, New York.*

Troelstra, C., A. van Gool, J. de Wit, W. Vermeulen, D. Bootsma, and J.H.J. Hoeijmarkers, (1992): *ERCC6,* a member of a subfamily of putative helicases, is involved in Cockayne's syndrome and preferential repair of active genes. *Cell.* **71**, 939-953.

Yoshinaga, S.K., C.L. Peterson, I. Herskowitz, and K.R. Yamamoto, (1992): Roles of SWI1, SWI2, SWI3 proteins for transcriptional enhancement by steroid receptors. *Science* **258**, 1598-1604

*Schizosaccharomyces pombe*에서 SNF2에 속하는 *hrp2+* 유전자의 특성 연구

최 인 순
신라대학교 생명과학과

본 연구는 분열형 효모 *Schizosaccharomyces pombe*에서 여러 가지 DNA 절제회복 및 유전자 발현에 관여하는 SNF2/SW12 유전자의 기능을 연구하기 위하여 이에 관련되는 유전자를 분리하고 그 특성을 연구하였다. SNF2 motif의 conserved sequence를 primer로 하여 중합효소 연쇄반응(PCR) 방법으로 480 bp 크기의 DNA fragment를 분리하여, 이를 probe로 하여 효모에서 *hrp2+* 유전자를 분리하였다. 분리한 *hrp2+* 유전자의 sequence homology를 비교한 결과 3개의 SNF2 motif를 포함하고 있었다. *hrp2+* 유전자의 전사체 크기는 4.7 kb임을 Northern hybridization으로 확인하였다. *hrp2+* 유전자의 전사 개시 부위를 알기 위하여 primer extension 분석을 한 결과, 첫 번째 ATG에서 약 47 base pair 위쪽에 위치함을 확인하였다. 또한 특성 연구를 위하여 Northern hybridization으로 *hrp2+* 유전자의 UV와 MMS에 대한 유도성을 조사한 결과 자외선에 대해서만 유전자의 발현이 유도되었다. 이 결과 분리한 *hrp2+*는 UV-inducible 유전자임을 확인하였다.