KOR. JOUR. MICROBIOL February, 1992 p. 37~41 Vol. 30, No. 1

Studies on *KEM1* Gene Controlling Mitotic Cell Division in Yeast: Molecular Cloning of a High Copy Suppressor (*ROK1*) of *kem1*

Kim, Sanghyeon and Jinmi Kim

Department of Microbiology, College of Natural Sciences, Chungnam National University, Daejeon 305-764, Korea

The *KEM1* gene is known to affect microtubule and spindle pole body function during the cell division cycle in *Saccharomyces cerevisiae*. To identify new genes with functions similar or related to those of *KEM1*, we isolated a high copy suppressor gene (*ROK1*) that suppresses the *kem1* mutation when cloned on a high copy number plasmid but not on a low copy number plasmid. Two clones which suppress both the benomyl hypersensitivity and the Kar$^-$ enhancing phenotype of *kem1* null mutation were isolated and were shown to have a 9.0 kb identical insert by restriction endonuclease analysis. The restriction map constructed indicates that this suppressor gene, *ROK1*, is not *KEM1*. Subcloning experiments suggest that the functional region of *ROK1* is at least 3.0 kb in size.

KEY WORDS □ ***KEM1*, cell division cycle, *Saccharomyces cerevisiae*, microtubules, spindle pole body, high copy suppressor**

The cell division cycle of the yeast *Saccharomyces cerevisiae* involves DNA replication, synthesis of cellular components, nuclear division, and cytokinesis (budding). The nuclear division is often described with the morphological changes of microtubules and spindle pole body (SPB, a microtubule organizing center of yeast) structures (3, 4, 14). Early in the cell cycle, SPB embedded in the nuclear envelope duplicates. As nuclear division proceeds, the two duplicated SPBs separate and migrate to the opposite sites of the nuclear envelope. Microtubules are formed between the two SPB and appear to assist the nuclear division process (7). Microtubules and SPB are also implicated in nuclear fusion of the conjugation cycle(3, 4). The two nuclei appear to move to each other by microtubules and fuse at the SPBs.

Several genes have been reported to be involved in microtubules and spindle pole body function. These include α-tubulin genes (*TUB1* and *TUB3*), β-tubulin gene (*TUB2*) (22, 17), *KAR1* (16), *KEM1* (10), and *CDC31* (16). *KAR1* gene is required for nuclear fusion and is most likely to be a component of the spindle pole body. *CDC31* controls spindle pole body duplication in response to the concentration of Ca^{2+} ion in the media (1).

KEM1 gene was identified by mutations which enhance the nuclear fusion defect of *kar1-1* mutation during conjugation (9, 10). *KEM1* appears to affect microtubule and spindle pole body function. Especially, *KEM1* controls chromosome transmission, nuclear division, and spindle pole body duplication/separation. *KEM1* was cloned and DNA sequence analysis showed that *KEM1* is a new gene with an open reading frame of 4.6 kb (10). Recently *KEM1* is found to be the same as several reported genes which were isolated by independent groups from different perspectives. These are *DST2*, *SEP1*, *XRN1*, and *RAR5*. *DST2* was isolated by purifying DNA strand trasferase activity *in vitro*. *XRN1* was by exoribonuclease I activity *in vitro* (Stevens, personal communication) and *RAR5* was isolated in a screen for genes involved in plasmid stability. Therefore, a great interest has focused on the primary *in vivo* functions of *KEM1*.

In the present study, to identify other genes with functions similar or related to those of *KEM1*, we isolated a high copy suppressor (*ROK1*) of the *kem1* mutation, which can suppress the *kem1* mutation when cloned on the high copy vector but not on the low copy vector. The restriction map of *ROK1* was constructed and the functional

This work was supported by a grant (No. KOSEF 903-0411-013-1) from the Korea Science and Engineering Foundation, Ministry of Science and Technology in 1990.

Table 1. *Yeast strains used in this study*

Strains	Genotype	Source
JK246	*MATa, ura3-52, his4-34, leu2-3,112 kem1Δ3::LEU2*	This Lab.
6942-1D	*MATa, leu2-3, lys1-1, met3*	G. R. Fink
7523-9D	*MATa, leu2-3, lys1-1,kar1-1 kar1-1*	G. R. Fink
SK201	JK246 [pSH1]	This study
SK202	JK246 [pSH2]	〃
SK250	JK246 [YCp50]	〃
SK260	JK246 [pJI60]	〃

size of this gene was estimated by subcloning experiments.

MATERIALS AND METHODS

Strains and media

The yeast *S. cerevisiae* strains used are listed in Table 1. *Escherichia coli* strain HB101 (2) was used for plasmid propagation. Yeast media and culture conditions were as described by Sherman *et al.* (19). YEPD contained 1% yeast extract, 2% peptone and 2% dextrose. YNB contained 0.67% yeast nitrogen base w/o amino acid and 2% dextrose. SC-Ura is a synthetic copmlete medium without uracil (YNB with all amino acids added except uracil). Benomyl medium was made by slowly adding a stock solution of benomyl (10 mg/ml in dimethyl sulfoxide, stored at −20℃) to warm YEPD with vigorous swirling to a final concentration of 15 μg/ml. Benomyl was a generous gift from E. I. Du Pont deMours and Co., Inc. Bacterial media were made as described by Maniatis *et al.* (12).

Transformation and DNA manipulation techniques

Yeast transformation was performed by the lithium acetate method (8) and *E. coli* transformation was by the method of Mandel and Higa (11). Plasmid DNA from *E. coli* was isolated by the modified method of alkaline lysis (12). Plasmids from yeast were obtained by isolating total yeast DNA and passaged through *E. coli*. Restriction endonuclease analysis and agarose gel electrophoresis were carried out as described in Maniatis *et al.* (12).

Coning of high copy suppressors

Yeast genomic library (from G. R. Fink laboratory) which was constructed on a high copy number vector YEp24 was used to isolate high copy suppressors of *kem1*. The *kem1* null mutant strain JK246 was transformed with the library DNA and was plated on the selective media, SC-Ura, in such a way that 200-300 transformants can appear on each plate. Colonies were replica-plated onto benomyl plates (15 μg/ml) and incubated at 26℃ for 1 day. Benomyl resistant colonies were considered as putative clones and streaked on benomyl plates. Ten transformants showed benomyl resistancy on the second test.

Plate mating assay

Patches of strains to be tested were grown on a YEPD plate and were mated to a lawn of strains with an opposite mating type and appropriate auxotroph markers. After incubation at 30℃ for 4 hr, plates were replica-plated onto YNB plates to select for diploids.

Plasmid constructions

To study the suppression activity of *ROK1* on a low copy plasmid, we constructed pSH57 by inserting 9.0 kb SalI-SmaI fragment of plasmid pSH1 into YCp50. To identify the functional region of *ROK1*, we cloned various restriction endonuclease fragments of the insert of the plasmid pSH1 into YEp24 plasmid. Plasmid pSH 56 was constructed by inserting 6.0 kb BamHI fragment into BamHI digested YEp24 and plasmid pSH12 was from self-ligation of BamHI digested pSH1. Plasmids pSH17 or pSH10 contained the 3.0 kb or 5.9 kb BglII fragment of *ROK1*, respectively, inserted into BamHI digested YEp24. Plasmid pSH30 was constructed by reclosing the remaining portion of the plasmid pSH1 after removal of 2.9 kb BglII fragment.

RESULTS

Isolation of a high copy suppressor of *kem1* mutation

To identify other genes with functions similar or related to those of *KEM1*, we investigated whether it is possible to isolate high copy suppressors of *kem1* which can suppress the *kem1* mutation when cloned on a high copy number plasmid. Yeast genomic library constructed on a high copy number vector YEp24 was transformed into the *kem1* mutant strain, JK246, and 10,000 transformants were obtained. One of *kem1* phenotypes is benomyl hypersensitivity, that is, antimicrotubule drug, benomyl, inhibits the growth of *kem1* mutants at concentrations (10-15 μg/ml) that do not affect the growth of wild-type strain (10). We screened the transformants by looking for those which suppressed the benomyl hypersensitivity and found ten putatives. To test whether these suppression phenotypes were originated from the plasmids, we cured the plasmids from ten candidates and observed the loss of the suppression activity. In two transformants, the suppression of the benomyl hypersensitivity cosegregated with the Ura marker of the plasmid. We also retrieved the plasmids from 10 colonies and retransformed back into the *kem1* strain. The suppression activity was again confirmed in the two clones, which is consistent with the above results from

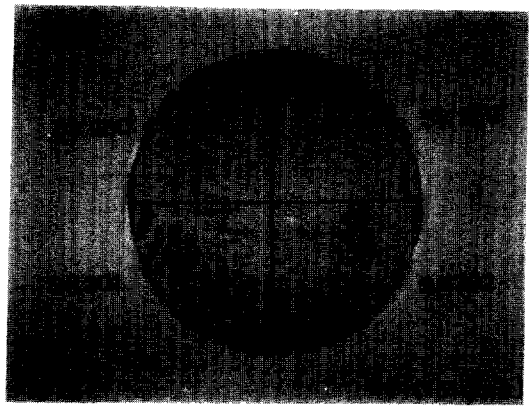

Fig. 1. *Suppression of the benomyl hypersensitivity of the kem1* mutation by high copy suppressors. *kem1 mutant strains transformed with a plasmid harboring KEM1 (SK260), vector alone (SK250), or high copy suppressor plasmids (SK 201, SK202) were streaked on YEPD media containing 15 μg/ml of benomyl. Plates were incubated for 2 days at 30°C and photographed.*

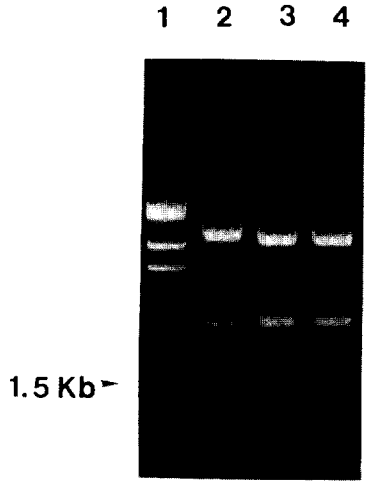

Fig. 2. *Restriction endonuclease analysis of ROK1 and KEM1. Plasmid DNAs of pSH1, pSH2, and pJI 98 were digested with BglII. Lane 1, HindIII size marker; lane 2, pJI98 (KEM1); lane 3 and 4, pSH1 and pSH2 (ROK1).*

the plasmid curing experiment. As summarized in Figure 1, *kem1* mutant (SK250) was sensitive to benomyl but *kem1* mutant strains transformed with either of the two newly isolated suppressor plasmids were resistant to benomyl (SK201 and SK202). As a positive control, the benomyl resistancy of *kem1* strain with *KEM1* on a plasmid was also shown (SK260).

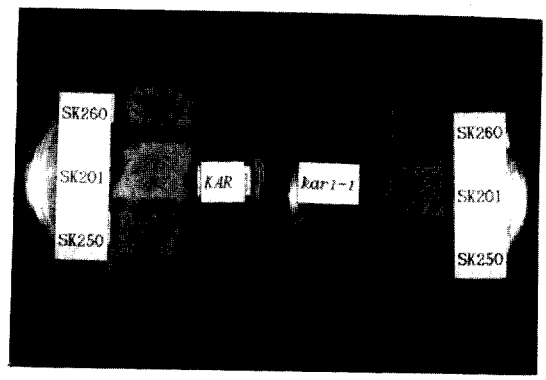

Fig. 3. *Suppression of Kar⁻ enhancing phenotype of kem1 mutation by the high copy suppressor ROK1. Patches of KEM1 strain (SK260), kem1 mutant strain with ROK1 on plasmid (SK201), and kem1 mutant strain (SK250) were grown on SC-Ura plate and were mated to a lawn of KAR or kar1-1 strains. After plate-mating, diploids were selected.*

Plasmids DNA were isolated from two suppressor colonies and were analyzed with several restriction endonucleases. Both plasmids had an insert of 9.0 kb and showed the same pattern of restriction fragments, indicating that the two are identical. To rule out that the *KEM1* gene complementing *kem1* mutation was recloned in our screening, we compared the restriction fragment patterns of the suppressor plasmid with those of *KEM1* plasmid. In the case of BglII digestion as an example, suppressor plasmids did not show 1.5 kb fragment that was seen in digestion of *KEM1* plasmid (Figure 2). These results suggest that a newly isolated high copy suppressor is not *KEM1* gene but a suppresor gene. This suppressor gene was named as *ROK1* (Rescuer Of Kem).

The high copy suppressor (*ROK1*) also suppresses the Kar⁻ enhancing phenotype of the *kem1* mutation

It was the benomyl hypersensitive phenotype of *kem1* that was used for the isolation of the high copy suppressor *ROK1*. We were interested in that *ROK1* also suppresses another crucial phenotype of *kem1*, *Kar* enhancing phenotype (*Kem*). *Kar* is a karyogamy defective (defective in the nuclear fusion during conjugation) phenotype of *kar* mutation and can be assayed by reduced diploid formation in crosses (5). *kem1* mutation enhances the nuclear fusion defect of *kar1* (10). Therefore, the frequencies of diploid formation in *kem1*×*kar1* crosses are much lower than those in *KEM1*×*kar1* crosses.

To examine the suppression of Kem phenotype by *ROK1*, we crossed *kem1* mutant strain carrying *ROK1* on a plasmid (strain SK201)

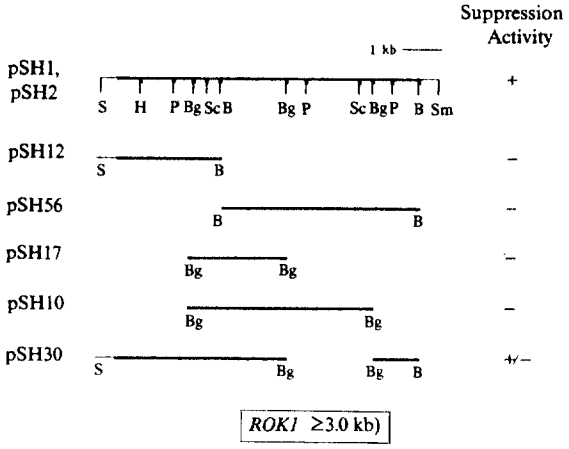

Fig. 4. *Restriction endonuclease map of the ROK1 gene and the subcloning plasmids. Restriction endonuclease sites are labelled as follows: B, BamHI; Bg, BglII; P, PvuII; S, SalI; Sc, SacI; Sm, SmaI. The suppression activities of the subcloning plasmids are indicated as + and −. The box at the bottom indicates the functional boundary of ROK1.*

to *KAR1* or *kar1-1* strains by a plate mating assay. As shown in Figure 3, in crosses to *kar1-1* strain, the strain SK201 showed the same degree of diploid formation as the positive control, *KEM1* transformant (SK260), whereas *kem1* mutant strain (SK250) did lower degree of diploid formation. These results demonstrate that the suppressor clone also suppresses the Kar enhancing phenotype of *kem1*.

The functional size of *ROK1* is at least 3.0 kb

To locate the functional boundary of *ROK1*, we constructed various subcloning plasmids and tested them for the suppression activity. As summarized in Figure 4, pSH10 and pSH56 did not show the suppression activity and pSH30 did a partial activity. Therefore, the functional region of *ROK1* appears to be at least 3.0 kb in size and includes two internal BglII sites. To investigate whether the *ROK1* gene has a suppression activity or not when cloned on the low copy vector, we inserted the SalI-SmaI fragment of 9.0 kb into YCp50, a yeast centromere plasmid. The *ROK1* gene on this low copy plasmid did not show the suppression activity.

DISCUSSION

We newly identified a gene, *ROK1*, which can rescue the defect of the *kem1* mutation. Since *ROK1* has the suppression activity on a high copy number vector but not on a low copy number vector, we assume that increased amount of *ROK1* gene products in cell can replace the *KEM1* functions. The *ROK1* gene might be a functional homolog of *KEM1* with weak activity. Another possibility is that *ROK1* could be functionally related to *KEM1* and be involved in the same cellular processes as *KEM1*. This type of suppression activity has been already reported in several other cases (6, 15, 21). For example, *cdc28* mutation is suppressed by overexpression of cyclin genes *CLB1*, *CLB2*, and *CLB4* in *S. cerevisiae* (21).

Benomyl phenotypes, either resistancy or hypersensitivity, have been considered as very specific to microtubule function. Both in *S. cerevisiae* and in *Aspergillus nidulans*, β-tubulin genes were identified as mutations conferring resistance to benomyl (22,18) and certain mutations in α-tubulin genes result in hypersensitivity to benomyl (17, 13). The fact that newly isolated *ROK1* suppresses the benomyl phenotype of *kem1* mutation suggests that *ROK1* also may affect the microtubule function as *KEM1* does.

REFERENCES

1. **Baum, P., F. Clement, and B. Byers,** 1986. Yeast gene required for spindle pole body duplication: homology of its product with Ca^{++} binding protein. *Proc. Natl. Acad. Sci.* U.S.A. **83**, 5512-5516.
2. **Boyer, H. and D. Roulland-Dussoix,** 1969. A complementation analysis of the restriction and modification of DNA in *E. coli*. *J. Mol. Bio.*, **41**, 469-472.
3. **Byers, B, and L. Goetsch,** 1974. Duplication of spindle plaques and integration of the yeast cell cycle. Cold Spring Harbor Symp. *Quant. Biol.* **38**, 123-131.
4. **Byers, B. and L. Goetsch,** 1975. The behavior of spindles and spindle plaques in the cell cycle and conjugation of *Saccharomyces cerevisiae*. *J. Bacteriol.* **124**, 511-523.
5. **Conde, J. and G. R. Fink,** 1976. A mutant of *Saccharomyces cerevisiae* defective for nuclear fusion. *Proc. Natl. Acad. Sci.* U.S.A. **73**, 3651-3655.
6. **Hadwiger, J. A., C. Wittenberg, M. A. de Barros Lopes, H. E. Richardson, and S. I. Reed,** 1989. A family of cyclin homologs that control G1 phase in yeast. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6255-6259.
7. **Huffaker, T. C., M. A. Hoyt, and D. Botstein,** 1987. Genetic analysis of the yeast cytoskeleton. *Ann. Rev. Genet.* **21**, 258-284.
8. **Ito, H., Y. Fukada, K. Murata, and A. Kimura,** 1983. Transformation of intact yeast cells with alkali cation. *J. Bacteriol.* **153**, 163-168.
9. **Kim, J.,** 1988. Genes controlling conjugation and mitotic cell division in yeast *Saccharomyces cerevisiae*. Ph.D. Thesis Massachusetts Institute of Technology, Cambridge.
10. **Kim, J., P. O. Ljungdahl, and G. R. Fink,** 1990.

kem mutations affect nuclear fusion in *Saccharomyces cerevisiae*. *Genetics* **126**, 799-812.
11. **Mandel, M. and A. Higa,** 1970. Calcium dependent bacteriophage DNA infection. *J. Mol. Biol.* **53**, 159-162.
12. **Maniatis, T., J. Sambrook, and E. F. Fritsch,** 1989. Molecular cloning 2nd ed. Cold Spring Harbor Lab. Cold Spring Harbor, N. Y.
13. **Oakley, B. and N. R. Morris,** 1980. Nuclear movement is -tubulin dependent in Aspergillus nidulans *Cell* **19**, 255-262.
14. **Pringle, J. R. and L. H. Hartwell,** 1981. The *Saccharomyces cerevisiae* cell cycle. pp.97-142 In Molecular biology of *Saccharomyces* vol.1 Cold Spring Harbor Lab. Cold Spring Harbor, N. Y.
15. **Richardson, H. E., C. Wittenberg, F. Cross, and S. I. Reed,** 1989. An essential G1 function for cyclin-like proteins in yeast. *Cell* **59**, 1127-1133.
16. **Rose, M. D. and G. R. Fink,** 1987. *KAR1*, a gene required for function of both intranuclear and extranuclear microtubules in yeast. *Cell* **48**, 1047-1060.
17. **Schatz, P. J., L. Pillus, P. Grisafi, F. Solomon, and D. Botstein,** 1986. Two functional α-tubulin genes of the yeast *Saccharomyces cerevisiae* encode divergent proteins. *Mol. Cell Biol* **6**, 3711-3721.
18. **Sheirr-Neiss, G., M. Lai, and N. Morris,** 1978. Identification of a gene for β-tubulin in *Aspergillus nidulans*. *Cell* **15**, 639-647.
19. **Sherman, F., G. R. Fink, and C. W. Lawrence,** 1979. Methods in yeast Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
20. **Stearns, T., M. A. Hoyt, and D. Botstein,** 1989. Yeast mutants sensitive to anti-microtubule drugs define three genes that regulate microtubule function. *Genetics*.
21. **Surana, U., H. Robitsch, C. Price, T. Schuster, I. Fitch, A. B. Futcher, and K. Nasmyth,** 1991. The role of *CDC28* and cyclins during mitosis in the budding yeast *S. cerevisiae*. *Cell* **65**, 145-161.
22. **Thomas, J. H., N. F. Neff, and D. Botstein,** 1985. Isolation and characterization of mutations in the β-tubulin gene of *Saccharomyces cerevisiae*. *Genetics* **112**, 715-734.

(Received December 31, 1991)
(Accepted January 22, 1992)

초 록: 효모에서 세포분열을 조절하는 *KEM1* 유전자에 관한 연구: *kem1*의 High Copy Suppressor (*ROK1*) 클로닝

김상현 · 김진미(충남대학교 자연과학대학 미생물학과)

*Saccharomyces cerevisiae*에서 *KEM1* 유전자는 세포분열시 microtubules 과 spindle pole body 구조체의 기능에 관여하는 것으로 알려져 있다. *KEM1* 과 유사하거나 연관이 있는 기능을 갖는 새로운 유전자들을 찾는 목적으로, *kem1* 돌연변이의 high copy suppressor 유전자, *ROK1*, 를 찾아 냈다. *ROK1* 은 high copy 플라스미드에 클로닝되었을 때 *kem1*을 suppression 하고, low copy 플라스미드에서는 suppression 하지 않는다. *kem1* 돌연변이의 benomyl 에 대한 민감성과 Kar enhancing 표현형을 동시에 suppression 하는 두개의 클론을 분리했으며, 제한효소로 분석했을 때 9.0 kb 의 insert 를 지닌 동일한 클론이었다. 이 suppressor 유전자 *ROK1* 의 제한지도를 작성하였고, 그 결과 *KEM1* 이 아닌 다른 유전자인 것으로 나타났다. Suncloning 실험으로 *ROK1* 은 적어도 3.0 kb 의 기능부위를 갖음을 확인했다.