Localization of *MAK*18 gene on chromosome VIII of *Saccharomyces cerevisiae*

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*Saccharomyces cerevisiae*의 염색체 VIII상의 *MAK* 18 유전자 국소화

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ABSTRACT: ***MAK18* gene of *Saccharomyces cerevisiae*, needed for M1 replication, was mapped within 2cM of *PET3* on chromosome VIII. From 38kb clone pRE66 carrying *SPO11* and *PET3*, we have localized *MAK18* gene whose insert is 2.8kb. *MAK18* gene is localized on about 9kb distance from *PET3* and about 18kb distance from *SPO11* on chromosome VIII.**

KEY WORDS□*MAK*18 gene.

Certain strains of *S. cerevisiae* secret a protein toxin that kills other strains, but the secreting strains are immune to the action of the toxin.

The genetic informations for the toxin production (K^+ phenotype) and resistance (R^+ phenotype) are present on an encapsidated linear double-stranded RNA molecule (1.8kb) denoted *M1*. *L-A* codes for the major coat polypeptide (81kdal) of intracellular virus-like particales in which both *L-A* and *M1* are found (Hopper *et al.*, 1977; Sommer and Wickner, 1982).

The maintenance of M1 ds RNA is also dependent upon at least 34 yeast chromosomal genes, namely 32 *MAK* (maintenance of killer) genes (*MAK1, MAK3-33* :Sommers and Bevan, 1969; Wickner, 1974a, 1978, 1979b; Wickner and Leibowitz, 1976a, 1979; Guerry-Kopecko and Wickner, 1980; Wickner, 1980; Wickner, 1986; personal communication), *PET18* gene (Leibowitz and Wickner, 1978), and *SPE2* gene (Cohn *et al.*, 1976). Because *MAK*18 gene is unknown but its avaibility was previously reported that pRE66 carrying *SP011, PET3,* and *SUF8* complemented *mak*18 mutation (Icho *et al.*, 1985).

To make profound researches on the role of *MAK* genes in *M1* ds RNA replication, we have localized *MAK18* gene from pRE66 and made the restriction map.

MATERIALS AND METHODS

Strains, plasmids, and Media

The strains and plasmids used are listed in Table 1. *E. coli* was used for maintenance, amplification, and isolation of plasmids and recombinant plasmids.

Media for cultivation of *E. coli* and *S. cerevisiae* were prepared according to Davis *et al.* (1980) Sherman *et al.* (1980), respectively. Solid media contained 1.5% Bacto-agar (Difco laboratories, USA). For the detection, ampicillin was added to LB media at a final concentration of 50 mg/*l*. The media used for cytoduction and assay for killer activity and resistance, are described in Table 2.

Table 1. *Yeast and bacterial strains and plasmids used.*

Strain	Genotype	Reference
S. cerevisiae		
3967	*a leu2, ura3, mak18,* HTF, [*KIL-K0*], ρ°	this work
1101	*α his4, kar-1,* [*KIL-K1*], ρ^{+}	this work
5×47	*a/α his1/+, trp1/+, ura3/+,* [*KIL-K0*]	killer-sensitive lawn, Wickner (1980)
E. coli		
HB101	F, *hsd20* (r^{-}B, r^{-}B), *recA13, ara-14, proA2, lacY1, galK2, rpsL20*(Sm^{r}), *xyl-5, mtl-1, supE44,* λ -	Boyer *et al.*, (1969)
Plasmid		
YCp50	*E. coli* and *S. cerevisiae* shuttle vector Amp^{r}, *URA3.*	Wickner (personal communication)
pRE66	38kb DNA fragment of *S. cerevisiae* chromosome VIII annealed into SalI site of YCp19 (Stinchocomb *et al.*, 1982) Amp^{r}, *URA3, PET3, SPO11.*	Elder and Esposito (personal communication)

Table 2. *The media composition (w/w)*

composition \ media	YPG	YPAD	MB(pH4.7)	S D	SD + Leu
Bacto-yeast extract	1%	1%	1%	—	—
Bacto-peptone	2%	2%	2%	—	—
Dextrose	—	2%	2%	2 %	2%
Bacto-agar	2%	2%	2%	2 %	2%
Glycerol	3%(v/v)	—	—	—	—
Bacto-yeast nitrogen base w/o amino acids	—	—	—	0.67%	0.67%
Leucine	—	—	—	—	0.003%
Methyleneblue	—	—	0.1%	—	—

* pH was adjusted to pH4.7 with 51.5% 0.2N NaH_2PO_4 and 48.5% 0.1N citrate buffer.

Yeast cells were grown at 37 °C at 80 rpm. Cell growth was monitored with a spectrophotometer (BECKMAN DU-7) on the basis of OD = 1.0 (2×10^7 cells/m*l*).

Cytoduction

A cytoplasmic genome can be transfered from one haploid strain to another without diplodization or other change of nuclear genotype by heterokaryon formaiton, using the *kar1* mutant which is defective in nuclear fusion (Conde and Fink, 1976). Recipient *mak18* mutant cells (strain No. 3967a) were [ρ°] (mitochondria defected cell by gowth on 5mg/m*l* ethidium bromide), and donor cells (strain No. 1101*α*) were [ρ^{+}] which have normal mitochondria. After mating recipient and donor cells on YPAD plates for 6hrs, the mating mixture was streaked on SD + Leu plates, on SD plates (for screening out diploid cells) and YPG plates (for screening out [ρ°] recipient cells).

Mitochondria of [ρ^{+}] cells which contained VLP (virus like particle) was used as a transfer marker of cytoplasmic factors to [ρ°] cells which included a *MAK18*-gene-carrying plasmids. Therefore, respiratory competent clones of [ρ^{+}]

cells having the recipient nuclear genotype-which grows on SD + Leu and YPG plates, but not on SD plate were the cytoductants needed in this experiment.

Enzymes and chemicals

Restriction endonucleases and T4 DNA ligase were purchased from BRL (Bethesda Research Laboratories, USA) or KOSCO Biotech. Lab. (Seoul, Korea). All reagents for DNA electrophoresis were obtained from BRL.

Restriction enzyme analysis

The plasmid DNA fragments produced by digestion with various restriction enzymes were separated by 1% agarose gel in TAE (0.04M Tris-acetate, 0.002M EDTA, pH8.0) buffer. The molecular size of each fragment was estimated by comparing distance of migration with phage DNA digested with HindIII. Fragments from double digestion were analyzed to determine the relative poistion of the cleavage sites in the insert.

Transformation

Cells of *E. coli* were transformed by the method of calcium choride (Maniatis *et al.,* 1982). Yeast cells were transformed by lithium ion method (Ito *et al.,* 1978).

Assay of killing and resistance

Cytoductant colonies were tested for killing ability by replica on MB plate (pH4.7) with a lawn of K1 killer-sensitive strain *S. cerevisiae* 5 × 47 (0.5 m*l* of 10^7 cells/m*l* suspension spread on plate and allowed to dry). MB plates were incubated at 20 °C for 2-3 days. In each case, killer activity was indicated by a clear zone surrounding the killer strains in the lawn of sensitive cells.

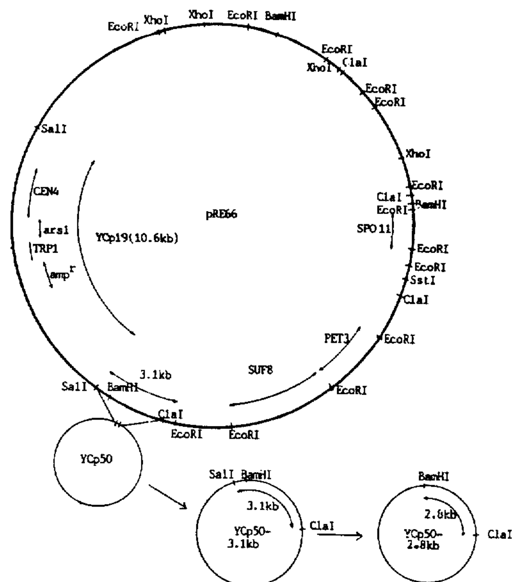

Fig. 1. *Localization of MAK18 gene activity in pRE66 and map of the 2.8kb ClaI/BamHI fragment containing the MAK18 gene.*
The activity of *MAK18* gene was localized by transforming strain 3967 (*a mak18, ura3*) with gel-purified 2.8kb restriction fragment of pRE66 annealed into ClaI/BamHI site of YCp50. The pRE66 carried *SPO11, PET3, SUF8* genes of *S. cerevisiae* chromosom VIII.

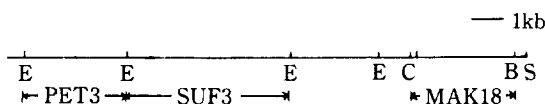

Fig. 2. *Restriction map near PET3 on chromosome VIII of S. cerevisiae Here we note the distance from PET3 to MAK18 is 9kb. E: :coRI, C:ClaI, S:SalI, st:SstI restiction sites are shown.*

RESULTS AND DISCUSSION

Using pRE66 deletion series, *MAK18* gene was cloned and identified by complementation test of *mak18* gene mutation, i.e., the recovery test of the ability to maintain K1-killer virus [*KIL-K1*] in *mak18* mutant cell. The *MAK18* gene was localized within the insert of gel-purified 3.1kb ClaI/SalI fragment from the pRE66, which was cloned into YCp50. (Fig. 1,3).

Progressive subcloning of the cloned 3.1kb DNA showed that *MAK*18 gene was within the 2.8kb ClaI/BamHI fragment (Fig. 3,5). Thus, this 2.8kb fragment was analyzed in YCp50 plasmid to determine the relative position of the restriction enzyme cleavage sites in the insert. The construction of the map was mainly facilitated by the various enzymes which cleave the plasmid at one, two, and in some cases three sites.

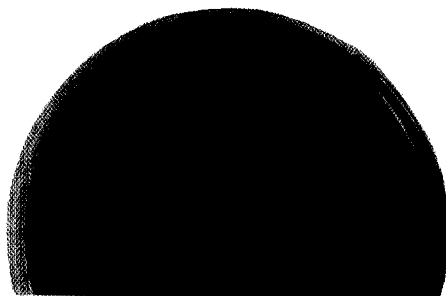

Fig. 3. *(below right) The activity of MAK18 gene by complement test.*
Subcloned plasmid is separately transformed into *S. cerevisiae mak18* strain (No. 3967*a*). Killer viruses are cytoduced into the cell by *S. cerevisiae* [*KIL-K1*] stain (No. 1101). Capabilities of maintenance of K1 killer are tested on killer toxin sensitive strain, *S. cerevisiae* 5 x 47 lawn by replica.
A: *S. cerevisiae* No. 3967 with 2.8kb BamHI/ ClaI fragment.
B: *S. cerevisiae* No. 3967 with 2.7kb BamHI/ SstI fragment.
C: *S. cerevisiae* No. 3967 with 3.1kb EcoRI/ BamHI fragment.

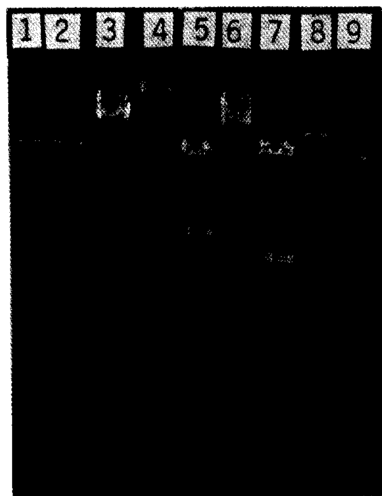

Fig. 4. *(above left) Restriction pattern of the recombinant plasmid in YCp50.*
Lane 4 contained size marker of phage DNA digested with HindIII. Double digestions of the recombinant plasmid inYCp50 with BamHI/ EcoRI (Lane 1), BamHI/ClaI (Lane 2), BamHI/ SalI (Lane 3), EcoRI/SalI (Lane 5), ClaI/EcoRI (Lane 6), BamHI/Sali/SstI (Lane 7), ClaI/SstI (Lane 8), BamHI/SstI (Lane 9).

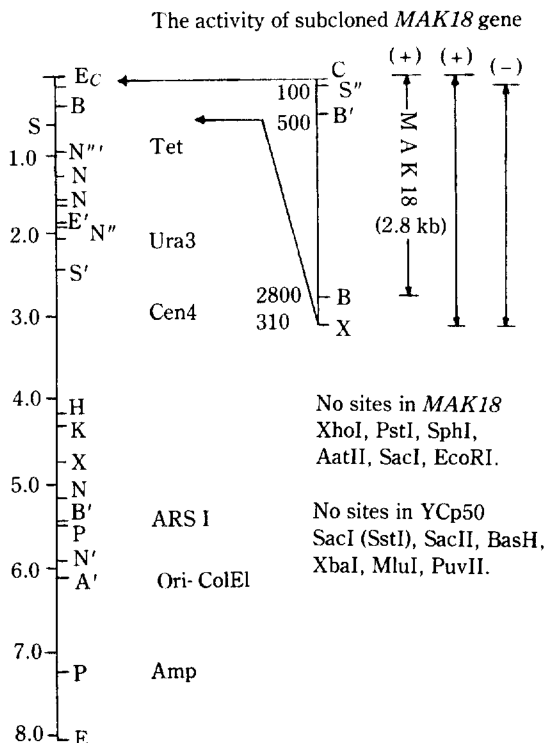

Fig. 5. *The activity of subcloned MAK18 gene in YCp50-3.1kb and map of the 2.8kb ClaI/BamHI fragment containing the MAK18 gene. MAK18 gene transforming activity was localized by trasforming strin 3967 with deleted YCp50-3.1kb. presence (+) or absence (−) of MAK18 gene activity is indicated.*
Restriction endonuclease recognition sites of *MAK18* gene region and vector YCp50 are shown; A:ApaI, A′:AflIII, B:BamHI, B′:BglII, C:ClaI, E:EcoRI, E′: EcoRV, H:HpaI, K:KpnI, N:NaeI, N′:NaeI, N″:NcoI, N‴: NruI, P:PstI, S:SalI, S′:SmaI, S″:SstI, X:XhI.

Digests of 2.8kb cloned in YCp50 plasmid revealed a single site for BamHI, ClaI, KpnI, PstI, SmaI, SstI, and XhoI; and two sites for BglII. Digests of YCp50 plasmid for contol revealed a single site for BglII, KpnI, PstI, SmaI, and XhoI, generating linear 8.05kb molecules. Therefore, in *MAK*18 gene, none of KpnI, PstI, SmaI, and XhoI restriction enzyme sites were present. There were SstI, BglI, and BamHI restriction enzyme sites. The distance of these sites from ClaI site was 0.1kb, 0.5kb, and 2.8kb, respectively (Fig. 4,5).

Comparing the sizes of the smallest and largest

subcloned plasmid of pRE66 deletion series, which complement *mak18* gene mutation, it was concluded that *MAK18* gene was located on 9kb distance from *PET3* gene and about 18kb distance from *SPO11* gene on the chromosome VIII in *S. cerevisiae* (Fig. 2., Esposito, personal communication). But *PET3* gene was known to be within 2cM (about 14-16kb) of *MAK18* in *S. cerevisiae* chromosome VIII (Mortimer and Schild, 1981).

Becuse map distance (or the "true" distance) between loci is not linearly related to recombinant frequency (RF) throughout the range of values possible in mapping experiments (Newlon, 1986), it is possible that there is about 5-7kb discrepancy. But the detail for this discrepancy has not been studied.

Further study of DNA sequencing analysis are to be necessary to elucidated the structural organization of *MAK18* gene including the accurate mapping of the restriction site, promoter site, and open reading frame to identify the sequence essential for its gene expression. It is also interesting the study of constructing genomic disruption of *MAK18* gene and gene fusions which place *lacZ* for transcriptional and translational control of *MAK18*. The genomic deletions and fusion plasmids should provide information on the function and control of *MAK18* gene. The gene integration test by the recombinant plasmid of *MAK18* gene-cloning YIp vector will be executed.

적 요

M1 복제에 필요한 *MAK* 18 유전자는 *S. cerevisiae*의 염색체 VIII상에 지도화 되었었다. *SPO* 11과 *PET* 3를 가지는 38 kb 클론의 pRE 66으로부터 *MAK* 18 유전자를 국소화 하여 최소 크기 2.8 kb와 같은 염색체의 다른 유전자인 *PETT* 3, *SPO* 11과의 거리를 밝혔다.

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