 © 1992 Oxford University Press

VDE endonuclease cleaves *Saccharomyces cerevisiae* genomic DNA at a single site: physical mapping of the *VMA1* gene

Meire C.D.Bremer[1,2], Frederick S.Gimble[1], Jeremy Thorner[1] and Cassandra L.Smith[1,2]*
[1]Department of Molecular and Cell Biology, Division of Biochemistry and Molecular Biology, University of California, Berkeley, CA 94720 and [2]Division of Chemical Biodynamics, Lawrence Berkeley Laboratory, Berkeley, CA 94720, USA

Submitted August 3, 1992

A DNA endonuclease, VDE, is derived from the *VMA1* gene product of the yeast *Saccharomyces cerevisiae* (1) and is related to other nucleases involved in nucleic acid rearrangements (2). Analysis of two cleavages sites showed that VDE recognizes an extended sequence, 5′=TATSYATGYYGGGTGY↓GGRG-AARKMGKKAAWGAAAWG-3′, and leaves a staggered double-strand break with 4-bp 3′-hydroxyl overhangs (1). Cleavage of one site in the *VMA1Δvde* allele (precisely deleted for the segment encoding VDE) during meiosis of a heterozygous diploid initiates a gene conversion event that transforms the mutant allele into a full-length *VMA1* gene (1). Here VDE cleavage of this same site *in vitro* was used to physically map the *VMA1* locus.

Genomic DNA containing the *VMA1Δvde* allele was isolated from *S.cerevisiae* strain YPH499-Δvde7D in agarose blocks (3), digested with VDE, and analyzed by pulse-field gel electrophoresis (PFG; 4). VDE cleaved chromosome IV quantitatively into two fragments of ~1.5 Mb (Figure 1A) and 150 kb (Figure 1B). These fragment sizes agree with the map position of the *VMA1* locus (Riles,L. and Olson,M., personal communication) on the far left arm of chromosome IV adjacent to the *SNF3* gene (5). Genomic DNA from an isogenic strain, YPH499 (6), containing the normal *VMA1* gene was not cleaved even at the highest VDE concentration added (Figure 1B).

Only 0.3 units of purified recombinant VDE (Gimble,F. and Thorner,J., manuscript in preparation) were needed to almost completely digest chromosome IV in 2 μg of total genomic DNA from YPH499-Δvde7D in a final volume of 200 μl [one unit cleaves 0.1 μg of plasmid pVMAΔvde (1) in 60 min at 30°C in 20 μl]. Similar results were obtained with VDE isolated from yeast (data not shown). VDE preparations were free of interfering nucleases because no non-specific DNA degradation was observed. VDE digestion conditions (Figure 1) are much simpler than those reported for another site-specific endonuclease with a large recognition site (7). VDE cleavage sites can uniquely mark specific genomic locations (8, 9). Thus, VDE is ideal for physical mapping of large genomes.

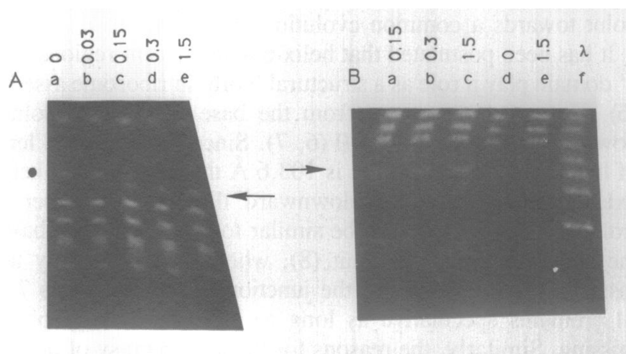

Figure 1. Site-specific cleavage of *S.cerevisiae* genomic DNA by VDE endonuclease. Genomic DNA (~2 μg) in agarose (0.5%) blocks was incubated with VDE at 30°C in 200 μl of 100 mM KCl, 25 mM Tris−HCl (pH 8.5), 2.5 mM $MgCl_2$, 2.5 mM 2-mercaptoethanol and 100 μg/ml bovine serum albumin for 4 hrs with gentle agitation, then deproteinized and fractionated by PFG as described (3) in a 1.1% agarose gel (LE, Seakem) in 0.8× modified Tris−borate buffer at 15°C using an inhomogenous electric field configuration. Enzyme added (in units) is indicated above each lane. **A.** VDE-digested DNA from YPH499-Δvde7D fractionated using a pulse program of 100 s (36 h), 600 s (36 h), 300 s (36 h) and 100 s (24 h) at a field strength of 5.5 V/cm. **B.** VDE-digested DNA from YPH499-Δvde7D (lanes a−d) and YPH499 (lane e) fractionated using a pulse program of 100 s (6 h), 60 s (8 h), 25 s (8 h), 5 s (8 h), and 1 s (6 h) at a field strength of 9.1 V/cm. Dot, intact chromosome IV; leftward arrow, 1.5 Mb fragment; rightward arrow, 150 kb fragment; and, λ (lane f), size standards (increments of 50 kb).

ACKNOWLEDGEMENTS

We thank Linda Riles and Maynard Olson for their help. This work was supported by Damon Runyon-Walter Winchell Cancer Research Fund postdoctoral fellowship #909 (to F.S.G.), by NIH Research Grant GM21841 (to J.T.), and by US Dept. of Energy contract #DE-AC03-76F00098 (to C.L.S.).

REFERENCES

1. Gimble,F.S. and Thorner,J. (1992) *Nature* **357**, 301−306.
2. Gimble,F.S. and Thorner,J. (1992) in Adolph,K.W. (ed.), *Methods in Molecular Genetics*. Academic Press, New York, in press.
3. Smith,C.L. *et al.* (1988) in, Davis,K. (ed.), *Genome Analysis: A Practical Approach*. IRL Press, Oxford, pp. 41−72.
4. Schwartz,D. *et al.* (1983) Cold Spring Harbor Symp. Quant. Biol. **47**, 18−23.
5. Link,A.J. and Olson,M.V. (1991) *Genetics* **127**, 681−698.
6. Sikorski,R.S. and Hieter,P. (1989) *Genetics* **122**, 19−27.
7. Thierry,A. *et al.* (1991) *Nucleic Acids Res.* **19**, 189−190.
8. Smith,C.L. and Kolodner,R. (1988) *Genetics* **119**, 227−236.
9. Smith,C.L. and Condemine,G. (1990) *J. Bacteriol.* **172**, 1167−1172.

* To whom correspondence should be addressed