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SELECTIVE INHIBITION OF YEAST KILLER-SPECIFIC DOUBLE-STRANDED RNA TRANSCRIPTION IN VITRO

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Summary: Virus-like particles were purified from three strains of Saccharomyces cerevisiae: the nonkiller, S7, the killer, A8209B, and the superkiller, T158C. RNA-dependent RNA polymerase activity copurified with the virus-like particles (ScV-L and ScV-M) from all three strains. The effect of elevated temperature (37° and 42°C) on the in vitro transcription reaction was investigated. Transcription of ScV-M showed a marked heat inhibition at 37°C and was completely inactive at 42°C. Transcription of ScV-L was unaffected by incubation at 37°C and exhibited a slight increase at 42°C. These results suggest that in vivo heat curing of the killer character may be due to a direct effect on the ScV-M viral RNA polymerase particle.

INTRODUCTION

Killer strains of Saccharomyces cerevisiae secrete a toxin which kills sensitive strains (1,2). The toxin and immunity are encoded by a cytoplasmically inherited species of double-stranded (ds) RNA 1.8 kbp in length designated M (3). In addition, nearly all strains of S. cerevisiae, killer or nonkiller, contain another ds RNA 4.8 kbp in length designated L (2). L and M are separately encapsidated into 30 nM virus-like particles designated ScV-L and ScV-M, respectively (4,5). L ds RNA encodes the viral coat protein (6).

An RNA-dependent RNA polymerase activity has been shown to copurify with ScV-L and ScV-M (7,8,9). The product of in vitro transcription of ScV-L is a 4.8 kb single-stranded (SS) RNA with homology to only one strand of L (9,10). The product of in vitro transcription ScV-M is a

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1.8 kb ss RNA with homology to only one strand of M (9). The transcripts of L and M are thought to function *in vivo* as messenger RNAs and may also serve as intermediates in the formation of new virus-like particles (VLPs) in a manner similar to the replication of reovirus (9,10,11).

Recessive mutations in any of 28 nuclear genes (MAK for maintenance of killer) results in the loss of ScV-M (2). A variety of treatments which are not mutagenic to chromosomal loci have been shown to cause the loss of ScV-M (12). These treatments include exposure to 5-fluorouracil (13), low concentrations of cycloheximide (14), and growth at 37°C (15). This phenomenon is referred to as "curing" of the killer character (2,12-15). There are two possible causes of the heat curing effect: 1) Heat could inactivate one of the MAK gene products. 2) Heat could exert a direct effect on the ScV-M particle itself.

In this investigation *in vitro* transcription of ScV-M was inhibited at 37°C and inactivated at 42°C. *In vitro* transcription of ScV-L was unaffected at 37°C and was stimulated at 42°C. These results suggest that *in vivo* curing of the killer character is due to a direct effect on the ScV-M particle.

MATERIALS AND METHODS

Growth of Yeast Strains

Strains S7, a nonkiller, A8209B, a killer, and T158C, a superkiller, were obtained from Dr. Gerald Fink. Strain 1556, which contains no ds RNA, was obtained from Dr. Reed Wickner. All strains were grown in complete medium as described by de Kloet et al. (16).

Isolation and Purification of Virus-Like Particles

Yeast cells were converted to protoplasts using snail enzyme (Beta-glucoronidase, Calbiochem.). Protoplasts were lysed in a French Pressure Cell (15,000 psi, one passage) and VLPs were purified as described by Welsh et al. (8). ScV-L and ScV-M were separated by sucrose density gradient centrifugation on a 5-20% (w/v) gradient in buffer G [50 m M Tris-HCl, pH 7.5, 10 m M $MgSO_4$, 1 m M dithiothreitol, 0.1 m M EDTA, 0.15 M NaCl, 0.39 M KCL, and 20% (v/v) glycerol]. The gradients were fractionated and assayed for RNA polymerase activity in the *in vitro* transcription reaction described below. Fractions containing purified ScV-L and ScV-M were pooled and the VLPs were pelleted by ultracentrifugation (133,000 xg). Purified ScV-L and ScV-M pellets were redissolved in 5 ml of Buffer G.

In Vitro Transcription

In vitro transcription of purified ScV-L and ScV-M particles was performed under the conditions described by Welsh et al. (8). Aliquots

of sucrose gradient purified VLPs were added to the reaction which contained 50 m M Tris-HCl (pH 7.4), 5 m M $MgCl_2$, 10 m M 2-mercaptoethanol, 0.1 m M EDTA, 20 m M NaCl, 5 m M KCL, 0.5 m M each of ATP, CTP, GTP, 20 μM [^{3}H] UTP (1.02 Ci/mmole), and 1.5 mg/ml bentonite in a final volume of 0.5 ml. The reactions were incubated at 30°, 37°, and 42°C for 3 hours. 50 μl aliquots of the reactions were placed on Whatman 3 M M filters presoaked in 10% TCA, 10 m M sodium pyrophosphate. The filters were washed with 10% TCA, air dried, and counted in 0.4% omnifluor in toluene in a Beckman liquid scintillation counter.

Purification of Ribosomal RNA

18S and 25S yeast ribosomal RNA for molecular weight standards were purified by sucrose density gradient centrifugation as described previously (17).

Electrophoretic Separation of RNA

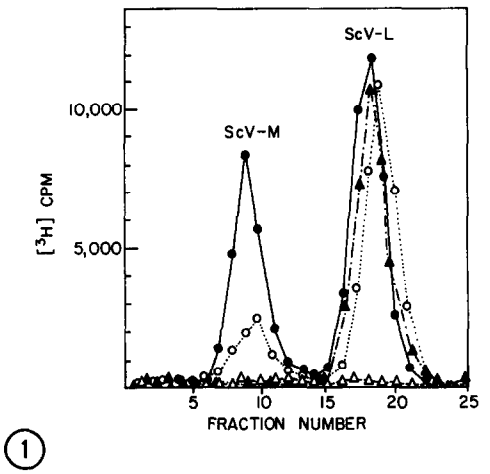
Following in vitro transcription ss RNA was extracted from the VLPs using proteinase K as described by Welsh et al. (8). The extracted RNA was precipitated with ethanol and redissolved in SSC. An equal volume of 4 M LiCl was added to precipitate high-molecular-weight ss RNA. The LiCl precipitate was dissolved in 6 M urea, 1 M sucrose, 0.25 M sodium citrate (pH 3.5). Samples were electrophoresed on 2% agarose tube gels containing 6 M urea, 0.25 M sodium citrate (pH 3.5) as described by Woo et al. (18). Following electrophoresis the gels were soaked in 0.1 M NaCl to remove urea. The gels were frozen and sliced with a Hoeffer Gel Slicer. The gel slices were counted in 0.4% omnifluor, 67% toluene, 33% Triton X-100.

18S and 25S ribosomal RNA were electrophoresed in parallel as molecular weight markers. The marker gel was stained with 0.5 μg/ml ethidium bromide for 30 min.

RESULTS AND DISCUSSION

VLP preparations from each of the four strains were subjected to sucrose density gradient centrifugation. The gradients were fractionated and each fraction was assayed for RNA polymerase activity as described in Materials and Methods (figure 1). Strain S7 exhibits only one peak of activity corresponding to ScV-L (S7 is a nonkiller and lacks ScV-M). Strains A8209B and T158C exhibit two peaks corresponding to ScV-L and ScV-M. The superkiller, T158C, exhibits more ScV-M activity than the normal killer, A8209B. This is in agreement with previous results which have shown that T158C contains more M ds RNA than normal killers (19). As expected, strain 1556, which does not contain ds RNA, did not exhibit RNA polymerase activity.

Following in vitro transcription ss RNA was extracted from the VLPs of strain T158C and subjected to agarose-urea gel electrophoresis (figure 2).



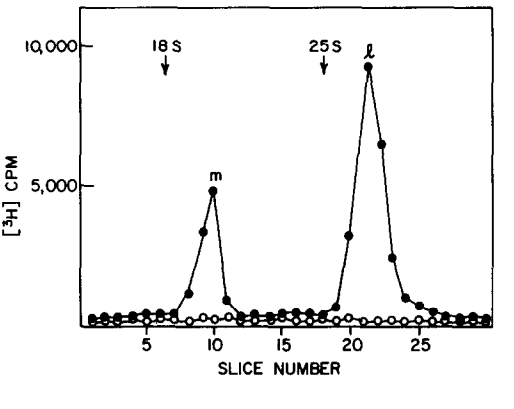


Figure 1. RNA polymerase activity in sucrose gradient purified VLPs from strains S7 (▲), A8209B (O), T158C (●), and 1556 (Δ). Fraction 1 is the top of the gradient.

Figure 2. Agarose urea gel electrophoresis of the transcripts from the VLPs of strain T158C (●) and the products of in vitro transcription of the extract from strain 1556 (O). The relative positions of the ethidium bromide stained 18S and 25S ribosomal RNA markers are indicated. Slice number 1 is the bottom of the gel.

The ^{3}H-labeled ss RNA (l and m) migrates with the molecular weight expected of full-length transcripts of L and M.

Table 1 shows the effect of elevated temperature on cell-free RNA synthesis in VLPs purified by gradient centrifugation. Both ScV-L and ScV-M exhibit RNA polymerase activity at 30°C (figure 1 and table 1). Production of l ss RNA remains constant at 37°C and shows a slight increase at 42°C. Single-stranded RNA synthesis (m) is strongly inhibited in ScV-M at 37°C and inactivated at 42°C.

Heat inactivation of a MAK gene product has been proposed as a mechanism for in vivo heat curing of the killer character. This hypothesis

Table 1. In vitro transcription of VLPs. 50 µl aliquots of each reaction were counted as described in Materials and Methods and background was subtracted.

	Counts per minute					
Reaction	L			M		
Temperature	T158C	A8209	S7	T158C	A8209	S7
30°C	7,076	6,701	6,776	2,803	921	-
37°C	7,485	6,709	6,823	393	35	-
42°C	7,942	6,830	6,864	0	0	-

seems unlikely because in vitro transcription of ScV-M is inhibited at 37°C while ScV-L is unaffected. These results suggest that heat curing of the killer character may be due to a direct effect on the ScV-M particle.

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REFERENCES

1. Bevan, E.A., Herring, A.J. and Mitchell, D.J. (1973) Nature 245, 81-86.
2. Wickner, R.B. (1979) Plasmid 2, 303-322.
3. Bostian, K.A., Hopper, J.E., Rogers, D.T. and Tipper, D.J. (1980) Cell 19, 403-414.
4. Herring, A.J. and Bevan, E.A. (1974) J. Gen. Virol. 22, 387-394.
5. Bruenn, J.A. (1980) Ann. Rev. Microbiol. 34, 49-68.
6. Hopper, J.E., Bostian, K.A., Rowe, L.B. and Tipper, D.J. (1977) J. Biol. Chem. 252, 9010-9017.
7. Herring, A.J. and Bevan, E.A. (1977) Nature 268, 464-466.
8. Welsh, J.D., Leibowitz, M.J. and Wickner, R.B. (1980) Nucleic Acids Res. 8, 2349-2363.
9. Welsh, J.D. and Leibowitz, M.J. (1980) Nucleic Acids Res. 8, 2365-2375.
10. Bruenn, J., Bobek, L., Brennan, V. and Held, W. (1980) Nucleic Acids Res. 8, 2985-2997.
11. Silverstein, S.C., Christman, J.K. and Acs, G. (1976) Ann. Rev. Biochem. 45, 375-408.
12. Wickner, R.B. (1976) Bacteriological Reviews 40, 757-773.
13. Bevan, E.A. and Mitchell, D.J. (1979) in Viruses and Plasmids in Fungi. Lemke, P.A., pp. 162-199, Marcel Dekker, New York.
14. Fink, G.R. and Styles, C.A. (1972) Proc. Natl. Acad. Sci. USA 69, 2846-2849.
15. Wickner, R.B. (1974) J. Bacteriol. 117, 1356-1357.
16. de Kloet, S.R., van Wermeskerken, K.A. and Koningsbergen, V.V. (1961) Biochim. Biophys. Acta 47, 138-143.
17. Mayo, V.S. and de Kloet, S. (1972) Arch. Biochem. Biophys. 153, 508-514.
18. Woo, S.L.C., Rosen, J.M., Liarakos, C.D., Cho , Y.C., Busch, H., Means, A.R., O'Malley, B.W. and Robberson, D.L. (1975) J. Biol. Chem. 250, 7027-7039.
19. Vodkin, M., Katterman, F. and Fink, G.R. (1974) J. Bacteriol. 117, 681-686.