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Relatedness of the Double-Stranded RNAs Present in Yeast Virus-Like Particles

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The relatedness of several double-stranded RNAs (dsRNA's) present in the virus-like particles of yeast was examined by T_1 fingerprint analysis. The dsRNA's examined were L, the dsRNA encoding the capsid polypeptide of yeast virus-like particles; M, which appears to code for a toxic polypeptide and for resistance to the effects of the toxin; and two S dsRNA's present in particles analogous to the defective interfering particles of animal viruses. S3, a dsRNA of 0.46×10^6 daltons, was derived entirely from M, a dsRNA of 1.2×10^6 daltons. S1, a dsRNA of 0.92×10^6 daltons, was a duplication of S3. This conclusion has also been reached independently by heteroduplex mapping techniques (H. M. Fried and G. R. Fink, personal communication). S1 and S3, at least in one yeast strain, were unstable in sequence, apparently due to the accumulation of sequence variants of the same molecular weight. L was a species of 3×10^6 daltons, unrelated in sequence to M, S1, or S3. S1, S3, and M had a 3′ T_1 dodecanucleotide in common.

Most strains of the yeast *Saccharomyces cerevisiae* harbor virus-like particles (VLPs) with double-stranded RNA (dsRNA) genomes (6, 10). These dsRNA's fall into three size classes (L, M, and S) named in analogy with reovirus dsRNA's. Yeast VLPs are inherited cytoplasmically, like many fungal dsRNA viruses lacking an infectious cycle. Little is presently known of their mode of replication, although a viral RNA-dependent RNA polymerase activity has recently been demonstrated (11).

The most common variety of yeast VLPs contains a dsRNA (L) of 2.5×10^6 to 3.0×10^6 daltons (4, 23, 25, 28). "Killer" strains, which secrete diffusible glycoproteins toxic to sensitive cells, have in addition VLPs containing a dsRNA (M) of 1.2×10^6 to 1.7×10^6 daltons (4, 7, 23, 25, 28, 30). VLPs containing L are separable from VLPs containing M either by sucrose velocity gradient centrifugation (10) or by cesium chloride equilibrium gradient centrifugation (D. Pietras and J. Bruenn, unpublished data). Particles containing L are isodiametic, about 35 nm across, with a density of 1.40 to 1.42 g/cm^3 (1, 10; Pietras and Bruenn, unpublished data). L, as demonstrated by in vitro protein synthesis, encodes the major polypeptide of L-containing particles (12). Genetic studies indicate that M probably encodes proteins necessary for killing and immunity to killer toxin. L and M would thus appear to be the genome of a multicomponent dsRNA virus similar to the known fungal dsRNA viruses (17). L and M may also be the genomes of two entirely independent dsRNA viruses, since there is as yet no evidence that L-containing VLPs have the same capsids as M-containing VLPs; furthermore, there are numerous nuclear genes affecting the maintenance of M that have no effect on the inheritance of L (27).

Killer strains sometimes give rise to "suppressive sensitive" strains, which harbor smaller (S) dsRNA's in place of M (22, 25). Haploid suppressive sensitive strains mated to haploid normal killers produce diploids whose meiotic segregants are invariably sensitive strains with S in place of M. S dsRNA's are also encapsidated in VLPs of the same density in CsCl as particles containing M (Pietras and Bruenn, unpublished data). The replication of S dsRNA's is apparently more efficient than that of M, since suppressive sensitive strains contain as much as 2 mol of S per mol of L, whereas killer strains have, at most, 0.2 mol of M per mol of L (25, 27; present paper). Thus, S-containing VLPs are probably defective-interfering particles (14). Killer factor has recently been reviewed in detail (18, 27).

We have previously shown that L and M each have only pppGp 5′ termini (5). In the present work, we establish some sequence relationships among these dsRNA's. L is without detectable sequence homology to M; two S dsRNA's are related to each other by a duplication and are entirely derived from M; the two S dsRNA's rapidly accumulate variants; and the one iden-

tified 3′ end of M is also present in both suppressive dsRNA's.

MATERIALS AND METHODS

Preparation of dsRNA. dsRNA uniformly labeled with $^{32}PO_4^{-3}$ to a specific activity of about 10^6 dpm/μg was prepared as previously described (5). Larger amounts (ca. 100 μg) of non-radioactive dsRNA were prepared from 10-liter batches of cells (ca 100 g [wet weight]), which were extracted with 2 volumes of 0.5% (wt/vol) sodium dodecyl sulfate–50% phenol in 50 mM Tris-chloride–1 mM EDTA (pH 7.5) for 3 h at room temperature. The resultant RNA was precipitated from 0.3 M sodium acetate with 2 volumes of ethanol. dsRNA was purified by CF11 cellulose chromatography (9) followed by 1.4% agarose or 3.5% polyacrylamide slab gel electrophoresis (see below). An agarose gel of [^{32}P]dsRNA from T132BNK3 is shown in Fig. 1. A small amount of single-stranded RNA (mainly tRNA as judged by its content of 2′-*O*-methyl-nucleosides) remained in the CF11 eluate and ran ahead of all the dsRNA. Bands (1 to 2 mm in width) were excised from gels, and the RNAs were eluted after homogenization of the gel slices.

Nuclease digestions. Complete T_1 digestions were performed, with an enzyme (Calbiochem)/RNA ratio of 1:20 (wt/wt), by heating the enzyme-RNA solution in 0.1× SSC (SSC = 0.15 M NaCl plus 0.015 M sodium citrate) in a closed capillary to 105°C for 3 min, adding the same amount of enzyme, resealing the capillary, and incubating for 6 h at 37°C. The double-stranded character of native, denatured and renatured dsRNA's was determined by digestion with pancreatic RNase or S1 nuclease. Pancreatic RNase digestions were in 2× SSC for 20 min at 25°C with 1 μg of enzyme per ml. S1 nuclease digestions (26) were in 0.25 M NaCl–0.03 M sodium acetate (pH 4.5)–1 mM $ZnSO_4$–5% (vol/vol) glycerol with 10 U of enzyme (Sigma) per ml at 45°C. Pancreatic RNase digestions of T_1 oligonucleotides eluted from DEAE thin-layer plates were performed as described previously (3).

FIG. 1. *Agarose gel (1.4%) electrophoresis of dsRNA's from T132BNK3. Autoradiography (1-h exposure) of dsRNA from cells labeled with 50 mCi of $^{32}PO_4^{-3}$. Some single-stranded RNA (mainly tRNA as judged by the content of 2′-O-methyl-nucleosides) is still present after elution from CF11 cellulose. These RNAs were cut out from narrow bands (1 to 2 mm), extracted from the gel by homogenization, and used for the fingerprints of Fig. 9 and 10.*

Homochromatography, electrophoresis, and analysis of T_1 fingerprints. Electrophoresis at pH 3.5 in 7 M urea on cellulose acetate strips (Schleicher and Schuell), transfer to DEAE-cellulose thin-layer plates (Brinkmann or Analtech), ascending chromatography in homomixture B or C, elution of T_1 oligonucleotides, and analysis of the pancreatic RNase products of T_1 oligonucleotides by electrophoresis at pH 3.5 on DEAE-cellulose paper (Whatman) were as described by Barrell (3).

Gel electrophoresis. Vertical slab gel electrophoresis was in slabs (17.5 by 17.5 by 0.4 cm) of 1.4% agarose or 3.5% acrylamide in E buffer (0.04 M Tris-hydrochloride, 0.005 M sodium acetate, 0.001 M EDTA [pH 7.8]). RNA was stained with ethidium bromide (1.0 μg/ml) in E buffer and photographed under UV illumination as described previously (20). Heat-denatured dsRNA was reacted with CH_3HgOH (10 mM, Apache Chemical Co.) before application to agarose gels run with EB buffer (0.05 M H_3BO_3, 0.005 M $Na_2B_4O_7 \cdot 10H_2O$, 0.01 M Na_2SO_4, 0.001 M Na_3EDTA [pH 8.19]). Gels of denatured RNA were stained in 0.5 M ammonium acetate with 1 μg of ethidium bromide per ml (2). We found that it is not necessary to include CH_3HgOH in the gels, but simply in the sample buffer, since the reaction with methylmercury is irreversible in the absence of complexing ions. This is not only a much cheaper procedure than that of Bailey and Davidson (2), but a more convenient one, since native and denatured RNAs (or DNAs) may be run side by side on the same gel. Reovirus dsRNA markers were a gift of R. Taber.

Electron microscopy. dsRNA (0.5 to 5 μg/ml) for electron microscopy was spread from a solution containing 2 mM EDTA, 10 mM Tris-hydrochloride, 50 μg of cytochrome *c* per ml, and 50% (vol/vol) formamide at a final pH of 8.5. Spreading onto a hypophase of distilled water, picking up the RNA on Parlodion-coated grids, and platinum-palladium shadowing were performed essentially as described by Davis et al. (8). Photographs were taken at a magnification of ×56,300 in a Hitachi HU11C electron microscope. Measurements of tracings of threefold enlargements were made with a map measurer. Simian virus 40 open circular DNA (a gift of J. Huberman) was included in some samples as a standard. Under these spreading conditions, simian virus 40 DNA had a contour length of 1.64 μm, and lambda DNA had a contour length of 16.5 μm.

Yeast strains. M dsRNA was prepared from strain T158DSK, which has about twice as much M as most killer strains. L was from T158DSK, S288C, T132BNK3, T132BNK1, or S7. S288C is a wild-type sensitive strain with only L. T132BNK3 and T132BNK1 are suppressive sensitive strains derived from an immunity-minus parent, T132B, that had both L and M, as well as S RNA of 0.5×10^6 daltons (23). S288C is from the collection of R. K. Mortimer, S7 was from C. McLaughlin, and the other strains were from the collection of G. R. Fink.

RESULTS

Molecular weights of the dsRNA's. The molecular weights of L and M have previously been estimated by polyacrylamide gel electrophoresis of dsRNA standards from *Aspergillus foetidus,* reovirus, or ϕ6 and by electron microscopy (4, 23, 25, 28). We measured the sizes of L and M and of the suppressive dsRNA's S1 and S3 by 1.4% agarose slab gel electrophoresis, with reovirus dsRNA standards on the same gel (Fig. 2). (S1 and S3 were from the suppressive sensitive strains T132BNK1 and T132BNK3, respectively [see above].) We arrived at values of 2.8×10^6, 1.3×10^6, 0.92×10^6, and 0.45×10^6 daltons for L, M, S1, and S3, respectively. Using these values for the molecular weights of the dsRNA's, as well as additional rRNA markers, we used the methylmercury denaturing gel system of Bailey and Davidson (2), which we modified to permit simultaneous electrophoresis of native and denatured dsRNA's on the same slab, to determine the molecular weights of the denatured forms of the above RNAs (l, m, s1, and s3). These values were 1.49×10^6, 0.62×10^6, 0.46×10^6, and 0.23×10^6 daltons (Fig. 3 and 4), or very close to half the molecular weights of the undenatured RNAs. Two forms of L (l1 and l2) were barely separable by this method (Fig. 4). Form l1 had some intrastrand secondary structure (J. Bruenn, in preparation). The molecular weight of l has previously been reported to be 1.5×10^6 (4). The base compositions of L, M, S1, and S3 were also consistent with two complementary strands of equal length (Table 1). The base composition of L (44.9% guanine plus cytosine) agreed well with previous estimates of 45% (4). The agarose gel size determinations are summarized in Table 2.

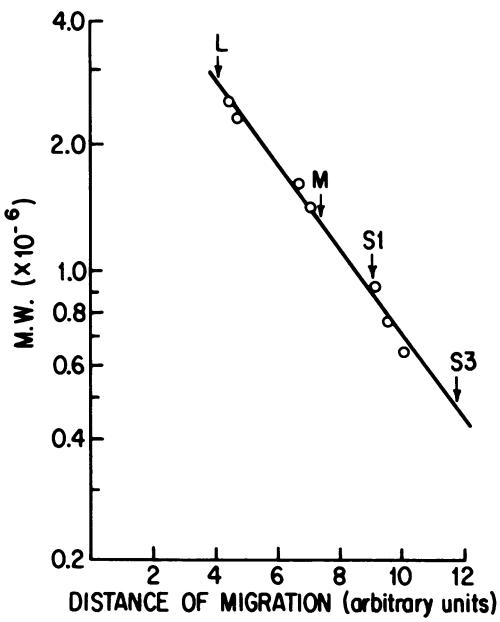

FIG. 2. *Electrophoresis of yeast dsRNA's with reovirus dsRNA markers. Reovirus dsRNA (a gift of R. Taber) was subjected to electrophoresis on the same 1.4% agarose slab gel with purified yeast dsRNA's. The positions of the differentiable bands of reovirus dsRNA's are the points shown, with arrows indicating the positions of yeast dsRNA's. Molecular weights of reovirus dsRNA's are from Shatkin et al. (21).*

The suppressive sensitive strain T132BNK3 was found to have many dsRNA's, but the major species present were L, S1, and S3 (Fig. 5). All putative dsRNA bands from the gel of Fig. 5 were verified as such by electron microscopy (not shown). S1 and the denatured S1 (s1) from T132BNK3 had the same molecular weights as the species present in the closely related strain T132BNK1 (Fig. 3). S1 (NK1) and S1 (NK3) from the original isolates also had the same T1 fingerprints (unpublished data), although this is probably not true of S1 (NK1) and S1 (NK3) from more recently cloned isolates (see below). All subsequent experiments were with S1 and S3 from strain T132BNK3.

Electron microscopy of L, M, S1, and S3 revealed primarily linear double-stranded forms such as those previously reported (23). Some grids included simian virus 40 open circular DNA as a marker, which had an average contour length of 1.64 μm. Assuming 2.3×10^6 daltons of dsRNA per μm (16), we arrived at molecular weights of 3.0×10^6, 1.2×10^6, 0.99×10^6, and 0.46×10^6 for the native forms of L, M, S1, and S3, respectively, in close agreement with values derived from agarose gel electrophoresis (Table 2).

The molecular weights of L and M were also estimated from 5'-end analysis. L and M have pppGp 5' termini (5). Assuming two such termini for each RNA, the molecular weight is given by $(320)\ (2)/[F_1/3) + (F_2/4)]$, where 320 is the average molecular weight of a nucleotide, F_1 is the fraction of total counts per minute present in ppGp and F_2 is the fraction of total counts per minute present in pppGp. The 5' termini were isolated after complete alkaline hydrolysis by

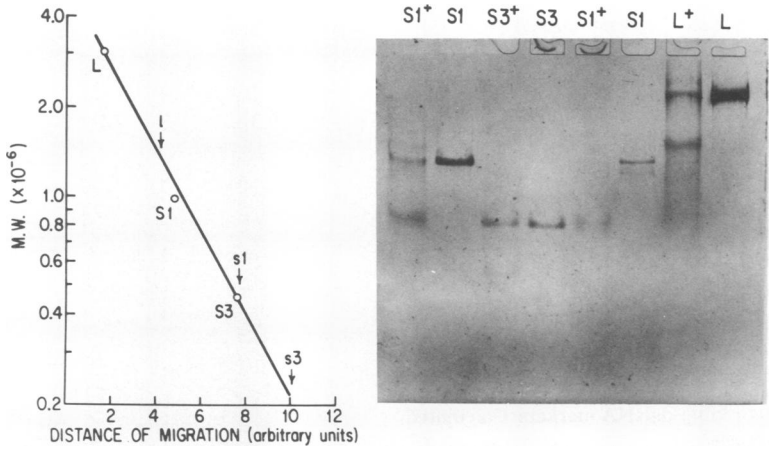

FIG. 3. *Electrophoresis of native and denatured yeast dsRNA's. The "+" signs indicate the addition of CH_3HgOH to denatured dsRNA's. Other samples are undenatured and without CH_3HgOH. The first two wells are S1 from strain T132BNK1; the remaining wells are of dsRNA's from T132BNK3. The band of denatured S3 is barely visible in this print, but its position is indicated on the accompanying drawing.*

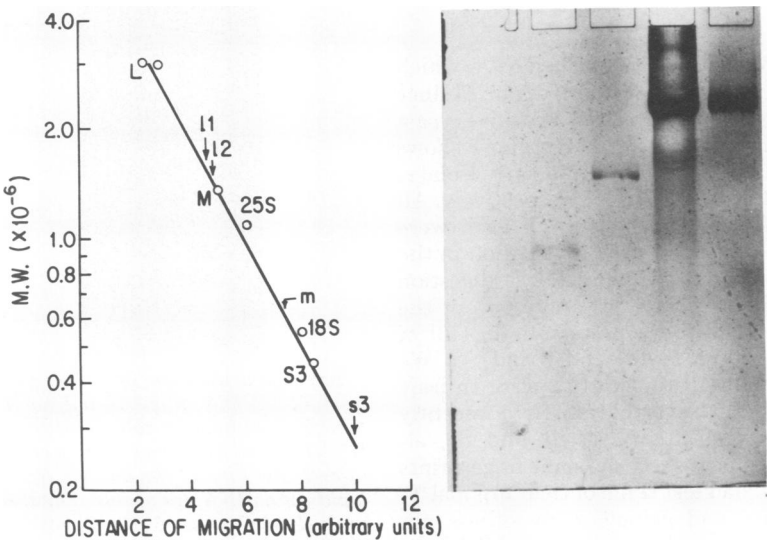

FIG. 4. *Electrophoresis of native and denatured yeast dsRNA's. As in Fig. 3, but the samples are (from left to right) denatured M, native M, denatured L, and native L (all from T158DSK). The positions of other RNAs on the same slab gel are shown in the accompanying drawing.*

TABLE 1. *Base composition of yeast dsRNA's*[a]

RNA	Percentage of:			
	C	A	G	U
L	22.7	27.8	22.2	27.2
M	20.2	30.6	20.3	28.9
S1	23.5	26.3	24.0	26.2
S3	22.8	25.9	23.6	27.5

[a] Averages of two to five experiments.

one-dimensional electrophoresis at pH 3.5 on DEAE-cellulose paper. Some hydrolysis of pppGp to ppGp occurred under these conditions. The molecular weights of L and M calculated in this manner are close to those estimated by electron microscopy or by gel electrophoresis (Table 2).

Sequence homologies. The T_1 fingerprints of L, M, S1, and S3 are shown in Fig. 6 to 10. None of these RNAs had a polyadenylic acid tract, as has previously been claimed (19). The pancreatic RNase digestion products of these T_1 oligonucleotides are given in Tables 3 to 6. None of the large T_1 oligonucleotides present in L was present in M (cf. Fig. 6 and 7, Tables 3 and 4). We also failed to detect homology between L and M by hybridization (not shown).

Although no homology was detectable be-

TABLE 2. *Sizes of yeast dsRNA's*

RNA	Size ($\times 10^6$ daltons)		
	Gel[a]	EM[b]	5′ Ends[c]
L	2.8	3.0	2.9
l	1.49		
M	1.3	1.2	1.1
m	0.62		
S1	0.88–0.95	0.99	
s1	0.46		
S3	0.45	0.46	
s3	0.23		

[a] Reo markers or killer dsRNA markers. Calculated by gel electrophoresis.

[b] Calculated by electron microscopy (EM), on the basis of 2.3×10^6 daltons of dsRNA/μg (16).

[c] Molecular weight = $(320 \times 2)/[(F_1/3) + (F_2/4)]$, where F_1 is the fraction of total counts per minute present in ppGp, and F_2 is the fraction present in pppGp.

tween L and M, the suppressive dsRNA's, which were originally postulated to derive from M since they replace it in suppressive sensitive yeast strains, did show homology to M. Figure 8 shows T_1 fingerprints of S1 and S3 from early isolates of T132BNK1 and T132BNK3, respectively. All of the large T_1 oligonucleotides of each were present in M, as judged both by position in the fingerprint and by pancreatic RNase digestion products (Table 5). Note that only two of the three T_1 oligonucleotides present in T_1 (10) of M were present in T_1 (5) of S1 and S3. We conclude that these two dsRNA's were entirely derived from M. Nevertheless, in a recently cloned isolate of T132BNK3, S1 and S3, although continuing to show the same fingerprints (Fig. 9 and 10), had lost some of their original T_1 oligonucleotides and gained many others not previously present or detectable in M (Table 6). Some 70 generations of growth intervened between the fingerprints of Fig. 8 and those of Fig. 9 and 10. T_1 fingerprints of S3 from isolates cloned at intermediate times were of intermediate complexity. We conclude that S3 accumulated variant molecules at a high rate (see below).

Since S1 always contained all the T_1 oligonucleotides of S3 and very few (if any) unique T_1 oligonucleotides and had twice the molecular weight of S3 (in unbroken single strands), S_1 must be a duplication of S3. The T_1 fingerprints of these two dsRNA's from the same cell line (T132BNK3) were always nearly identical regardless of how much they diverged from the original (Fig. 9 and 10). Note that the one identified 3′ end of S3 was present in S1 at lower

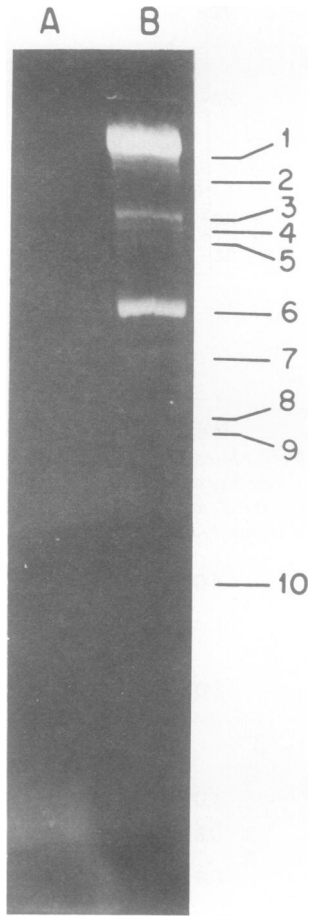

FIG. 5. *Purification of non-radioactive dsRNA's from T132BNK3 on a 3.5% polyacrylamide slab gel. Well A contained a sample of the single-stranded RNA fraction from the cellulose column; well B contained one-third of the dsRNA fraction. At least 10 bands of dsRNA are visible. The three major bands are 1 (L), 3 (s1), and 6 (S3). The 25S and 18S rRNA's remained in the phenol-treated cells.*

molarity than in S3 (cf. Fig. 9 and 10). This is precisely what would be expected if S1 were a duplication of S3. The same 3′ end was present in M, S1, and S3. The absence of a 3′ terminal G on this T_1 oligonucleotide was verified by snake venom phosphodiesterase digestion. The 3′ terminal residue is pC_{OH} (Bruenn, unpublished data). We conclude that S3 probably arose from one end of M, or by internal deletion of M (H. Fried and G. R. Fink, personal communication), and was subsequently duplicated to produce S1.

Since S1 and S3 from the same cells remained essentially identical in large T_1 oligonucleotides while diverging from the original S3 sequence, most of the S1 present in these cells must have derived from duplication of S3 in every genera-

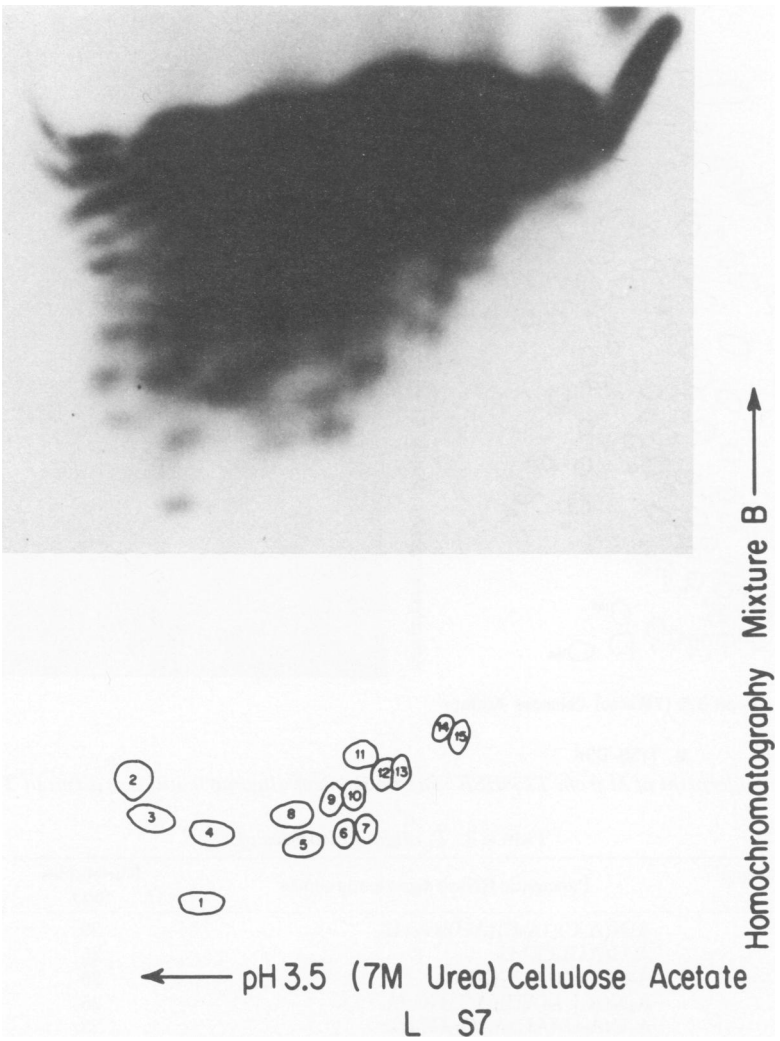

FIG. 6. *T_1 fingerprint of L from S7. On the right is an autoradiograph of a T_1 fingerprint of L, as described in the text. The numbers of the T_1 oligonucleotides in the accompanying drawing correspond to those in Table 3. This fingerprint was performed with homochromatography mix B, whereas those for Fig. 7 to 10 were performed with homochromatography mix C.*

tion, rather than from one initial event. In the latter case, S3 would diverge from S1 present in the same cells, which it did not. A consequence of this argument is that the S1 present in strain T132BNK1, also derived from T132B, but lacking the smaller dsRNA S3 present in T132BNK3, should have diverged from the S1 and S3 present in T132BNK3. We are in the process of investigating this prediction.

T_1 fingerprints of L from S7, T158DSK, and T132BNK3 were identical with respect to all analyzed large T_1 oligonucleotides. This fact is consistent with the interpretation that the genotypic differences among these strains lie in M.

DISCUSSION

We found that the two dsRNA's present in VLPs in wild-type killer yeast strains (L and M) were unrelated. Suppressive sensitive strains of yeast had smaller dsRNA's encapsidated in VLPs; these smaller dsRNA's were derived from M and rapidly accumulated sequence variants. They are similar to the RNAs (or DNAs) of defective interfering particles of animal viruses.

Work is now in progress in our laboratory on the comparison of M species from different strains and of suppressive dsRNA's from a number of independent isolates. We would like to

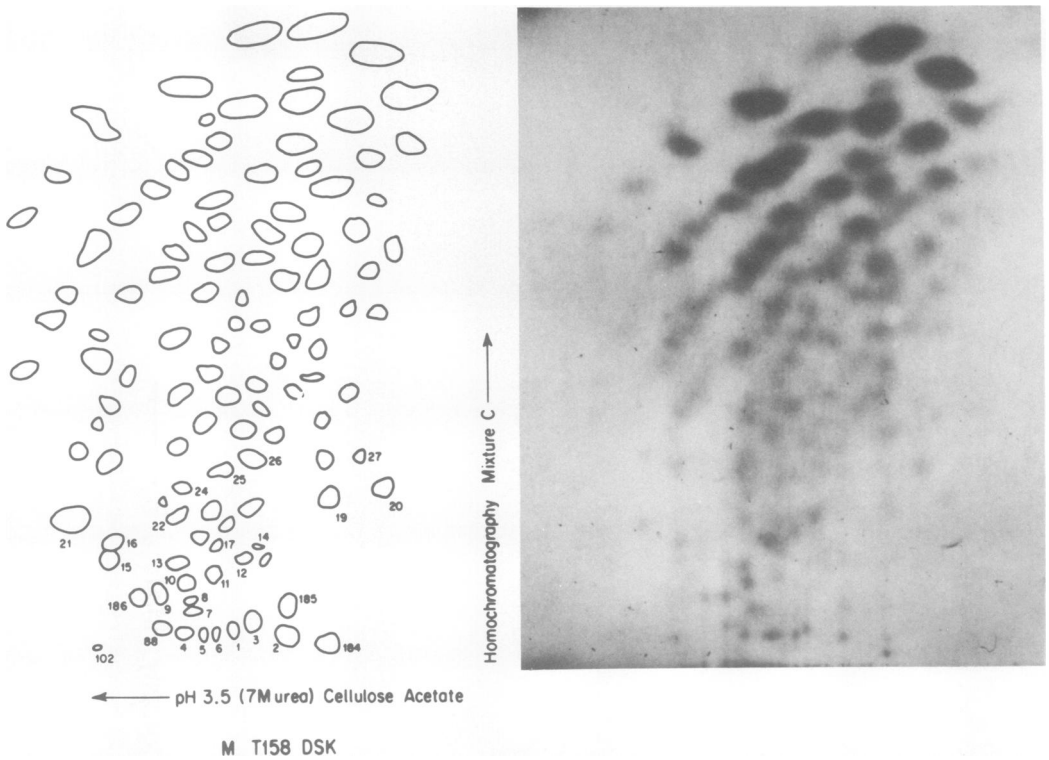

FIG. 7. *T_1 fingerprint of M from T158DSK. The analysis of oligonucleotides is given in Table 4.*

TABLE 3. *T_1 oligonucleotides of L*[a]

Oligonucleotide spot no.	Pancreatic RNase digestion products	Nucleotides (no.)	Length
1	$A_4N(A_3C)_2(A_2C)(AU)_4GC_6U_8$	39	39
2	$A_2U(AU)_4GC_4U_9$	25	25
3	$A_3U(A_2U)(AU)_2GC_2U_6$	20	20
4	$A_4N(A_2U)_2(AU)(AC)_2GC_3U_4$	25	25
5	$A_2U(A_2C)(AU)_3(AC)_2GC_5U$	23	23
6 + 7	$A_4N(A_3U)(A_3C)(A_2C)(AG)_2(AU)(AC)_8C_8U_4$	50	25
8	$(A_2C)(AU)_4(AC)_3(A_2G)C_7U_4$	31	31
9	$A_2G(A_2C)(AU)(AC)_3C_4U_3$	21	21
10	$A_3C(AC)_3GC_6U_4$	20	20
11	$(A_3G)(A_2C)(AU)_2(AC)_5GC_9U_5$	36	18
12 + 13	$A_4N(A_3C)_2(AG)(AC)_5C_8U_4(A_2G)$	40	20
14	$A_2C(AG)(AC)_2(AU)C$	12	12
15	$AG(AC)_3C_3$	11	11

[a] Two experiments.

know whether the event(s) that resulted in the duplication of S3 in S1 reported here, and confirmed by electron microscopy as a tandem duplication (Fried and Fink, personal communication) occurs often and whether the 3′ end of the M present in S1 and S3 is present in other suppressive dsRNA's. If so, S1 and S3 would be similar to the RNAs of vesicular stomatitis virus defective interfering particles, which have 3′ ends identical to those of their parental RNA. Vesicular stomatitis virus RNAs also have 5′ pppPu (purine) ends and will form circles in vitro, as have been observed for the dsRNA's S1 and S3 (Bruenn, in preparation; 15).

We believe that the increased complexity with time of S3 and S1 of strain T132BNK3 is not artifactual. It is reproducible from experiment to experiment and does not appear to be due to contaminating RNAs: the same contaminating species would have to be present in each case,

TABLE 4. *T_1 oligonucleotides of M*[a]

Oligonucleotide	Pancreatic RNase digestion products	Nucleotides (no.)	Length
1	$C_6U_5(AG)(AC)_3(A_2C)(AU)_3$	28	28
2	$(AC)_3(A_2C)_2(A_3G)(A_3C)$	20	20
3	$C(AC)_3(AG)(AU)(A_2U)(A_2C)_2$	20	20
4	$C_2U_4(AC)_2(AU)_2(A_2U)(AG)$	21	21
5	$C_4U_4(AG)(AC)_3(AU)(A_2C)_2$	24	24
6	$C_4U_3(AG)(AU)_2(A_3C)(AC)$	19	19
7	$GU_2(AU)(A_2U)(AC)(A_4C)$	15	15
8	$C_3U_4(AG)(AU)(AC)_2(A_2U)$	18	18
9	$GC_2U_4(AC)(A_2U)_2(AU)_4$	23	23
10	$GC_3U_8(AG)_2(AC)_3(AU)_4(A_2U)_3(A_3C)$	43	15
11	$GC_2U_4(AC)_2(A_2U)$	14	14
12	$G(AC)(AU)_2(A_2C)(A_3C)$	14	14
13	$GC_3U_3(AG)(AC)_3(AU)_6(A_2U)$	30	15
14	$(A_3G)(A_4C)_2$	14	14
15	$GU_4(AC)(AU)(A_3U)$	13	13
16	$GC_3U_5(AC)(AU)_2$	15	15
17	$GC_3U_2(AC)(AU)_2(A_2U)$ + $GCU_3(AC)_3(AU)_2$	30	15
19	$G(AC)(A_2C)(A_3C)$	10	10
20	$(A_3C)(AC)_2(AU)C$	11	11
21	$GC_5U_7(AG)(AC)_3(A_2C)$	24	12
25	$GC_5U_3(AG)(A_2G)(AU)_2(AC)(A_2U)(A_2C)(A_3C)$	30	10
27	$C_3(A_2G)(AC)_3(A_2C)$	15	15
102	$GC_3(U)(AU)_5(A_2C)(A_2U)_2$	17	17
186	$GC_2U_5(AC)_3(AU)(A_3U)$	20	20

[a] Five experiments.

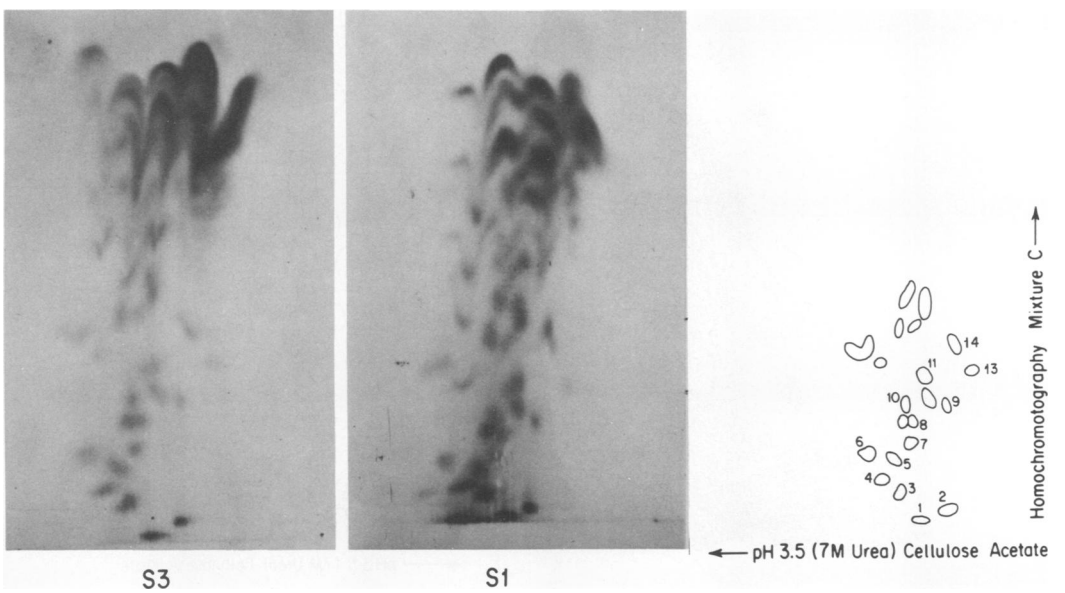

FIG. 8. *T_1 fingerprints of S1 and S3 from the original suppressive non-killer isolates T132BNK1 and T132BNK3. The pancreatic products of these T_1 oligonucleotides are shown in Table 5.*

since the fingerprints of S1 and S3 are essentially identical. Since S3 migrates some 3 cm ahead of S1 on gels from which 1-mm slices are taken, this seems highly unlikely. The more likely explanation is that S3 accumulates sequence variants and that S1 (in the same cells) is usually the result of duplication of S3 rather than of autonomous replication.

L apparently lacks the 3′ end present in M, S1, and S3. This may reflect different mecha-

TABLE 5. *T_1 oligonucleotides of S3 and S1 from early-cloned isolate*

Oligonucleotide spot no. of:		Pancreatic RNase digestion products	Nucleotides (no.)	Length (*N*)
S3 or S1	M			
1	1	$C_6U_5(AG)(AC)_3(AU)_3(A_2C)$	28	28
2	3	$C(AG)(AU)(AC)_3(A_2C)_2(A_2U)$	20	20
3	8	$C_3U_4(AG)(AU)(AC)_2(A_2U)$	18	18
4	9	$GC_2U_4(AC)(AU)_4(A_2U)_2$	23	23
5	10	$C_2U_6(A_2U)_2(A_3C)(AU)(AC)_3(AG)_2$	31	15
7	11	$GC_2U_4(AC)_2(A_2U)$	14	14
8	17	$GC_3U_2(AC)(AU)_2(A_2U)$	15	15
9	14	$(A_3G)(A_4C)_2$	14	14
11	25	$GC_5U_3(AC)(AU)_2(AG)(A_2G)(A_2U)(A_3C)(A_2C)$	30	10
13	20	$C(AC)_2(AU)(A_3C)$	11	11
14	27	$C_3(A_2G)(AC)_3(A_2C)$	15	15

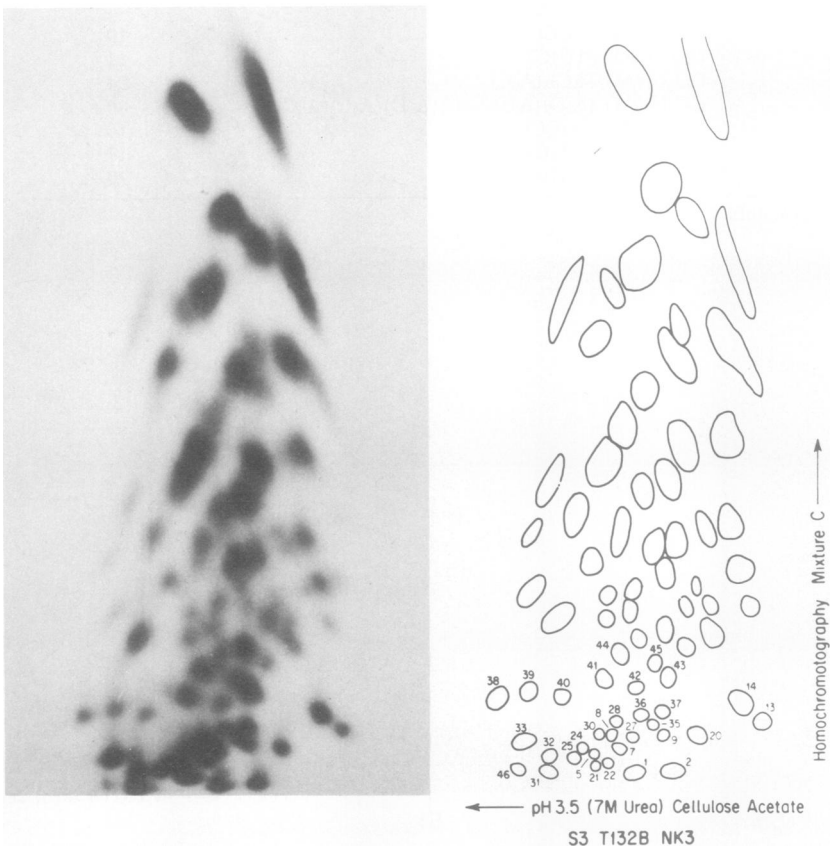

FIG. 9. *T_1 fingerprint of S3 from a cloned isolate of T132BNK3 70 generations after the original isolation. The analysis of these oligonucleotides is given in Table 6. The homomixture C used in this experiment and that of Fig. 10 was subjected to a somewhat longer KOH treatment than that of Fig. 7 and 8.*

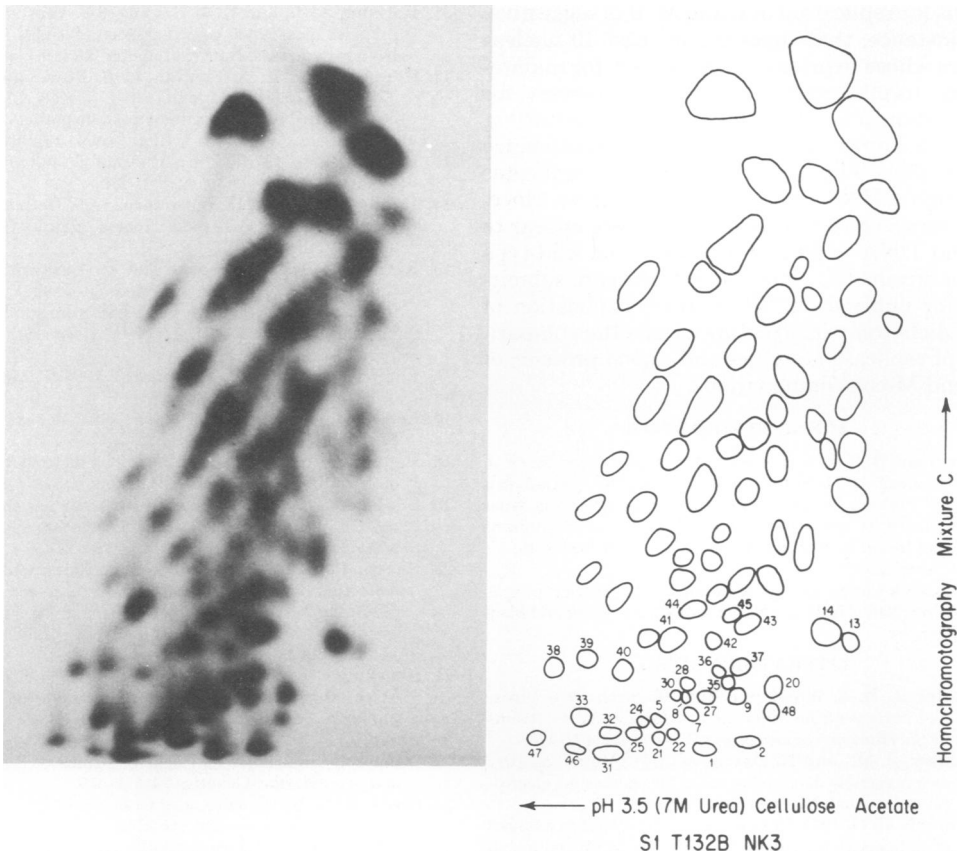

FIG. 10. *T_1 fingerprint of S1 from the same experiment as the S3 of Fig. 9. The analysis of T_1 oligonucleotides is given in Table 6.*

TABLE 6. *T_1 oligonucleotides of S3 and S1 from recently cloned isolate*[a]

Oligonucleotide spot no. of:		Pancreatic RNase digestion products	Nucleotides (no.)	Length (*N*)
S3 or S1	M			
1	1	$C_6U_5(AG)(AC)_3(AU)_3(A_2C)$	28	28
2	3	$C(AG)(AU)(AC)_3(A_2C)_2(A_2U)$	20	20
5	10	$C_2U_6(AG)_2(A_2U)_2(A_3C)(AU)(AC)_3$	30	15
7	11	$GC_2U_4(AC)_2(A_2U)$	14	14
8	17	$GC_3U_2(AC)(AU)_2(A_2U)$	15	15
9	14	$A_3G(A_4C)_2$	14	14
13	20	$C(AC)_2(AU)(A_3C)$	11	11
14	27	$C_3(A_2G)(AC)_3(A_2C)$	15	15
20		$C_3(AU)(AC)_2(A_2G)$	12	12
22		$GC_2U_2(AU)(A_2U)(A_4N)$	15	15
24		$U_2(AG)(AU)_2(AC)_2(A_2U)$	15	15
25		$GCU_3(AU)_2(AC)(A_2U)_2$	17	17
27		$GC_4U_2(AU)_3(AC)$	15	15
28		$GC_3U_2(AU)_3(A_2U)$	15	15
30		$GC_2U_3(AU)_2(AC)_2$	14	14
31		$C_2U_4(AG)(AC)_2(AU)_4$	20	20
32		$CU_3(AC)(AU)_2(AG)(A_2U)$	15	15
33		$GC_2U_3(AC)(AU)_2(A_2C)(A_3U)$	19	19

[a] One experiment.

nisms for replication of L and M. It is suggestive, for instance, that there are at least 10 nuclear genes whose expression is necessary for maintenance (replication) of M but unnecessary for replication of L (27). At least one of these nuclear genes is known to be required for maintenance of S (22). The possibility that L replicates through a DNA intermediate is unlikely, since, contrary to a recent report (24), there appear to be no DNA copies of L present in wild-type killer strains (N. Hastie and J. Bruenn, submitted for publication; 28). Further explication of this dichotomy in structure awaits the comparison of replicase activities and capsid proteins of L- and M-containing virions.

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