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Evolution of Defective-Interfering Double-Stranded RNAs of the Yeast Killer Virus

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We have characterized by T_1 fingerprint analysis several defective interfering (DI) double-stranded RNAs of the simple yeast virus ScV. A common sequence of about 0.5 to 0.6 kilobase pairs, including both 3′ termini of the parental RNA, was present in each DI RNA. Several DI RNAs had novel T_1 oligonucleotides not present in their parental RNA.

Saccharomyces cerevisiae has a simple double-stranded (ds) RNA virus (ScV) with two, separately encapsidated dsRNA's (4, 10). Like other fungal viruses (17) ScV exhibits only a latent infection. The larger viral dsRNA (L) encodes the major capsid protein (14). The smaller (M) encodes a toxin ("killer factor") lethal to strains without ScV-M (*S. cerevisiae* virus-M-containing) particles (1, 16; K. A. Bostian et al., Cell, in press). L and M have no detectable sequence homology (2), and ScV-M particles are dependent on ScV-L particles for the synthesis of their capsid protein (8, 11). Deletion mutations of M result in defective-interfering (DI) particles containing fragments of M (ScV-S particles) (2, 7; this work). ScV-M and ScV-S particles are equally dependent on products of the nuclear genome for their replication, products not necessary for the replication of ScV-L particles (19, 20). ScV-S RNAs are derived from M by internal deletion, sometimes followed by tandem duplication (2, 7), and they diverge in sequence from their parental RNA (2), as do the RNAs of animal viruses replicated in persistent infections (13).

In the present work we utilize two-dimensional gel electrophoresis of T_1 oligonucleotides to characterize several S RNAs, including three independently derived S RNAs from parental strains with different genetic backgrounds. The dsRNA's were phenol extracted and purified by CF11 chromatography and agarose gel electrophoresis (2, 3). The sizes of dsRNA's were determined by electron microscopy and by CH_3HgOH agarose denaturing gel electrophoresis (2). The two new S dsRNA's examined were S14, of 0.78 kb (kilobase pairs), from strain L014 (M. Vodkin); and S733, of 0.77 kb, from strain K733 (R. Wickner). The S dsRNA's were present in ScV particles with a density in CsCl of 1.36 g/ml. ScV-L particles had a density of 1.42, and ScV-M particles had a density of 1.37 g/ml.

We have fingerprinted four of these dsRNA's: S1(a) from strain T132B NK3; S1(b) from strain T132B NK1; S14, and S733. These are compared with M from strain T158D SK and with a previously published T_1 fingerprint of S1 from strain T132B NK3, S1(c). Uniformly $^{32}PO_4^{-3}$-labeled dsRNA was purified and digested with RNase T_1 as previously described (2, 3), and the products were separated by two-dimensional polyacrylamide gel electrophoresis (5, 6). The resolution of the two-dimensional gel system is greater than that of the cellulose acetate-homochromatography system previously used (2). We have adopted a new numbering system for both S and M. Figures 1 and 2 depict only the resolvable large T_1 oligonucleotides, and Table 1 gives the pancreatic digestion products of those large T_1 oligonucleotides of M present in one or more S dsRNA's.

All four S dsRNA's are clearly derived from M. S1(a), -(b), and -(c) have a minimum of 19 large T_1 oligonucleotides of M; S14 and S733 have 17. Each dsRNA has both of the 3′ T_1 oligonucleotides of M (oligonucleotides 5 and 13 or 54 of S and 43 and 13 or 44 of M). Therefore, all appear to be the result of internal delection of M, followed by tandem duplication in the case of S1 (2, 7). Three of the four S dsRNA's have large T_1 oligonucleotides not present in M. Of those novel T_1 oligonucleotides previously observed in S1 [2, shown in S1(c)], S1(b) has preserved all three (no. 3, 8, and 20) and added two more (the two T_1 oligonucleotides in spot 4). S1(a) has lost all three. S1(b) has lost one of the 21 T_1 oligonucleotides originally derived from M [no. 44 of S1(a) or S1(c)].

S14 is slightly larger than S3 (50 to 100 base pairs), but it clearly arose from M in an event very similar to that which generated S3. S14 has

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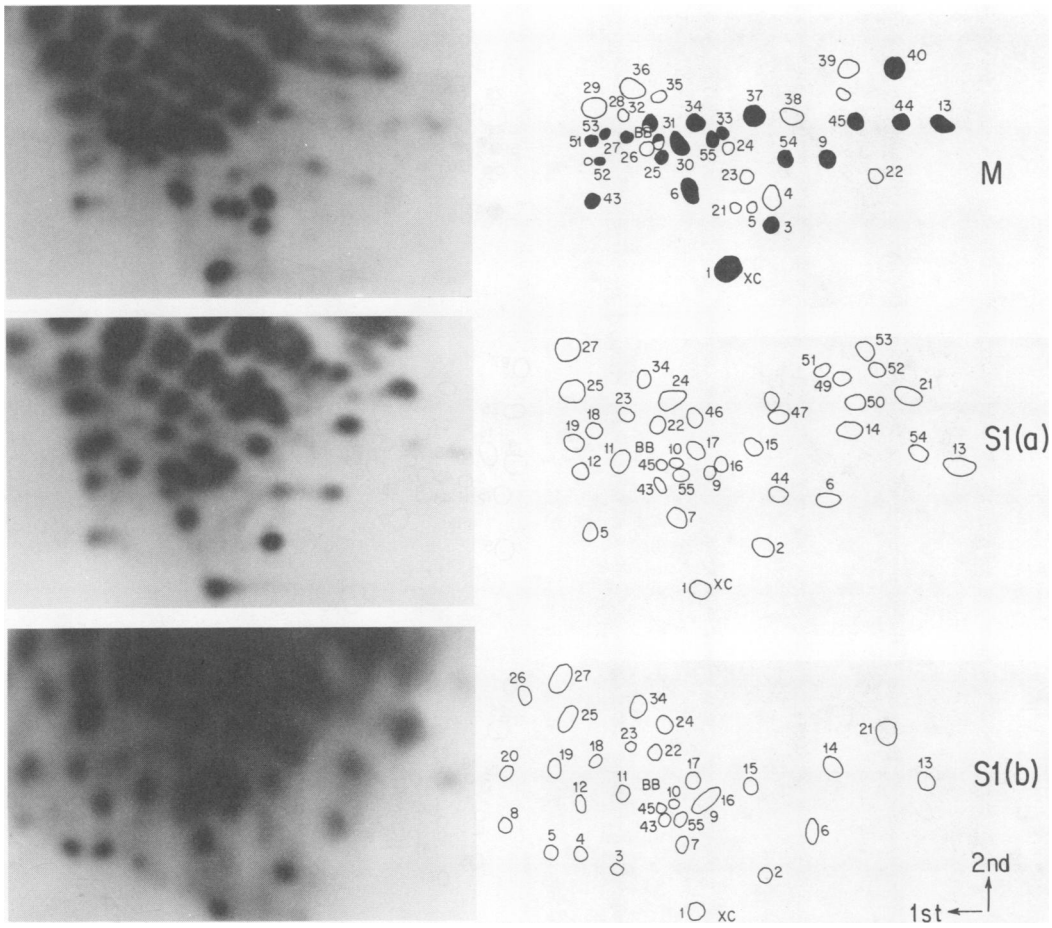

FIG. 1. *Two-dimensional gel electrophoresis of T_1 digests of $^{32}PO_4^{-3}$-labeled dsRNA's. Refer to Table 1 for the pancreatic analysis of the T_1 oligonucleotides. The positions of the bromophenol blue (BB) and the xylene cyanol (XC) marker dyes are shown for each gel. T_1 oligonucleotides of M that are present in one or more S dsRNA's are shaded. (S1(a) is S1 dsRNA from strain T132B NK3. S1(b) is S1 dsRNA from strain T132B NK1.*

17 of the 21 large T_1 oligonucleotides from M present in S1(c). It has both of the 3′ T_1 oligonucleotides of M. In contradistinction to S1, it has no detectable large T_1 oligonucleotides not derived from M. S733, on the other hand, has at least three large T_1 oligonucleotides not present in M (no. 56, 62, and 65). S733 is similar in size to S3, preserves both 3′ ends of M, and contains 16 of the 21 large T_1 oligonucleotides derived from M by S3. S733 has also been observed to form tandem duplicates, that is, some clonal isolates of strain K733 produce an S RNA of twice the usual size with the same T_1 fingerprint. S733 has one additional large T_1 oligonucleotide (no. 63) derived from M (no. 32) that is not present in any other S dsRNA so far fingerprinted.

The existence of the same 3′ termini on M, S1, S33, and S14 has been confirmed by 3′ end labeling and sequence analysis (V. Brennan and J. Bruenn, unpublished data). The T_1 fingerprints of in vivo labeled RNA have demonstrated the heterogeneity of at least one 3′ end of M; T_1 oligonucleotides 13 and 44 of M have different mobilities but apparently have the same pancreatic digestion products (Fig. 1 and 2 and Table 1). This would be consistent with a change in the 3′ terminal nucleoside, which is not detected in the pancreatic digestion products. Most of the S dsRNA's have the 3′ terminal T_1 oligonucleotide with the mobility of 13, but S733 has one with the mobility of 44 (no. 54 of S733). S1(a) has both, like M. The other 3′ terminus is tentatively assigned to T_1 oligonucleotide no. 5 of S (no. 43 of M). The pancreatic products and mobility of this oligonucleotide are

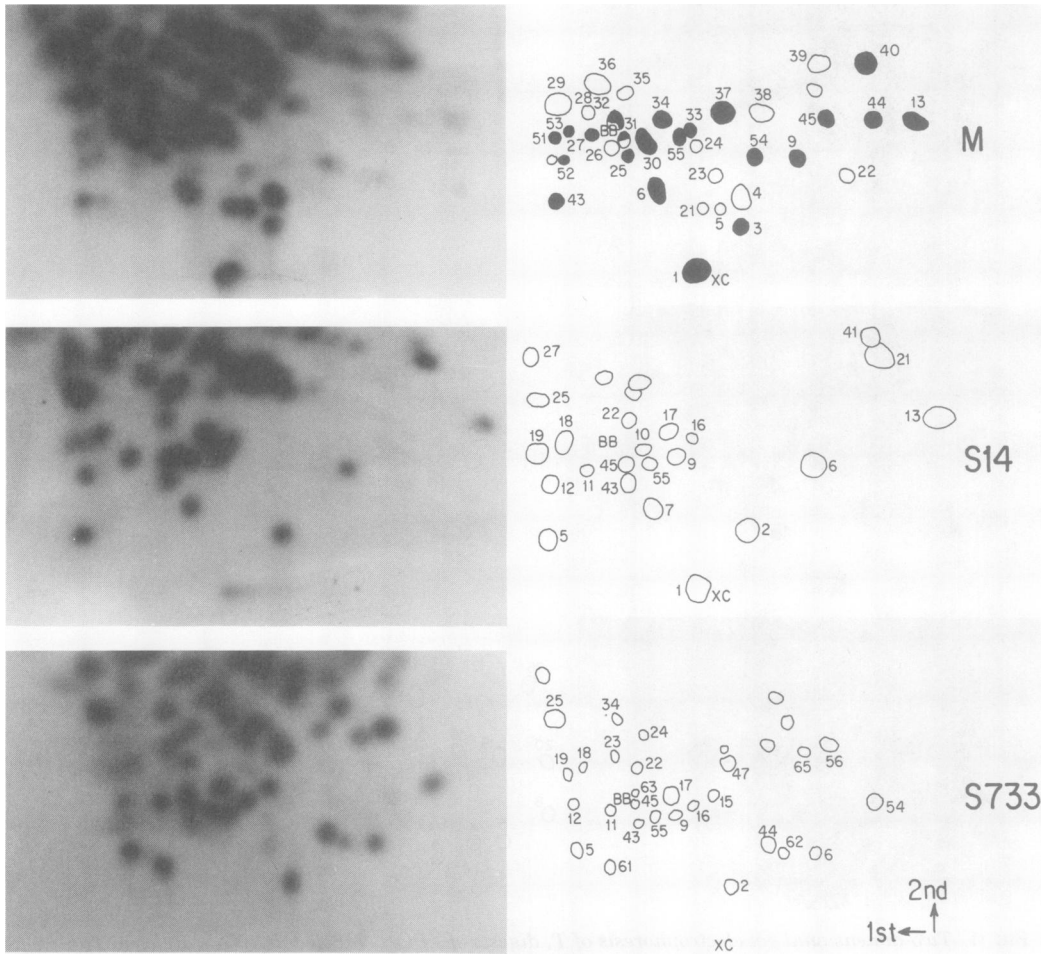

FIG. 2. *T_1 fingerprints of S733 and S14 are compared with the same M T_1 fingerprints as those in Fig. 1. Notation is as explained in Fig. 1.*

consistent with the 3′ sequence analysis (Brennan and Bruenn, unpublished data). S733 appears to have two possible 3′ T_1 oligonucleotides at this end (no. 5 and 61) with closely related sequences.

Among the four DI RNAs analyzed, of which three are independently derived, there is a core of some 12 large T_1 oligonucleotides from M that are present in each S (Table 1), including both 3′ T_1 oligonucleotides of M. Each of the S dsRNA's thus appears to be the result of the internal deletion of M, as suggested for S3 (7). Furthermore, this deletion seems to occur in a roughly similar place in each case. The complexity of the original S dsRNA derived from M can, in each case, be approximated by the expression $C = N/(0.24)(1\text{–}0.24)^{n-1}$, where N is the number of T_1 oligonucleotides of size n or larger and 0.24 is the frequency of G residues (2). This figure is about 0.65 to 0.80 kb in each case, which agrees well with the size of S3, S14, and S733 (0.7 to 0.8 kb). The core sequence corresponds to about 0.5 to 0.6 kb. There also appears to be a size constraint in the formation of DI RNAs, since there are only the two size classes: that of S3 and that of S1, the latter being a tandem duplication of the former. At least some animal virus DI RNAs are also generated by deletion (15).

A simple model can account for both deletion and tandem duplication. ScV RNA replication appears to be similar to that of reovirus (9, 12, 18; J. D. Welsch and M. J. Leibowitz, personal communication). If a transcriptase copying M dissociated from its template, reassociated at some internal sequence, and then completed synthesis, a deletion would result. If the transcriptase completed the synthesis of the viral plus strand and dissociated from its template

TABLE 1. *T_1 oligonucleotides of M and defective interfering dsRNA's*[a]

S1(a)	S1(b)	S1(c)	S14	S733	M	Pancreatic RNAse products
1	+	NR	+	−	1	$(AG)(AU)_5(AC)_5C_2U_7$
2	+	+	+	+	3	$(A_2C)(AG)(AU)_2(AC)_3C_4U_3$
−	3	+	−	−	−	$(AG)(AU)_4(AC)_2C_2U_4$
−	4	−	−	−	−	$(A_3C)(A_2U)(AG)(AU)(AC)_2GC_4U_6$
5	+	+	+	+	43	$(AU)_{2\text{-}3}C_3U_{8\text{-}10}$
6	+	+	+	+	9	$(A_2U)(A_2C)_2(AG)(AU)(AC)_2C$
7	+	NR	+	−	6	$(A_2U)(AG)(AC)C_6U_6$
−	8	+	−	−	−	$(A_2U)(A_2C)(AG)(AU)_2(AC)CU_3$
9	+	+	+	+	55	$(A_2U)(AC)_2GU_2C$
10	+	+	+	−	30	$(AU)_2(AC)_2GC_2U_3$
11	+	+	+	+	27	$(A_2U)(AG)(AU)_2(AC)CU_3$
12	+	+	+	+	52	$(A_3U)(AU)(AC)GU_4$
13	+	+	+	−	13	$(A_3C)(AU)(AC)_2C$
14	+	+	−	−	45	$(A_2G)(AU)(AC)_2C_3$
15	+	+	−	+	37	$(AC)_4GC_3U_2$
16	+	+	+	+	33	$(AU)_3(AC)_2GC_5U_2$
17	+	+	−	+	34	$(A_2U)(AU)_2(AC)GC_4U_2$
18	+	+	+	+	(53)	$(AU)_3(AC)GC_2U_5$
19	+	+	+	+	51	$(AU)_2GC_3U_8$
−	20	+	−	−	−	$(AU)_2GC_2U_4$
21	+	+	+	−	40	$(A_2G)(A_2C)(AC)_3C_3$
43	+	+	+	+	25	$(A_2U)(A_2C)(AU)_2(AC)GCU_3$
44	−	NR	−	+	(54)	$(A_2U)(A_2C)_2(AG)(AU)(AC)_2C$
45	+	+	+	+	31	$(A_2U)(AG)(AU)(AC)_3CU_3$
54	−	−	−	+	44	$(A_3C)(AU)(AC)_2C$
55	+	+	+	+	30	$(A_3C)(A_2U)(AG)C_2U_3$
−	−	−	−	56	−	$(A_2C)(AG)(AC)_3C$
−	−	−	−	61	−	$(AU)_3(AC)C_3U_8$
−	−	−	−	62	−	$(A_3C)(A_2C)(AC)_3G$
−	−	−	−	63	32	$(AU)_4(AC)GC_3U_3$
−	−	−	−	65	−	$(A_3C)(A_2C)(AG)(AC)_2C$

[a] T_1 oligonucleotides are numbered as in Fig. 1 and 2. Symbols: +, present; −, absent; NR, not resolved; ( ), pancreatic RNAse products not tested. S1(c) is S1 dsRNA analyzed on 10 April 1977 from T132B NK3 (2). S1(b) is S1 dsRNA analyzed on 1 June 1978 from T132B NK1. S1(a) is S1 dsRNA analyzed on 1 December 1978 from strain T132B NK3. S14 is S14 dsRNA from strain L014. S733 is S733 dsRNA from strain K733, and M is M dsRNA from strain T158D SK.

without releasing the plus strand, the transcriptase could then associate with a second molecule of S present in the particle (or with the same molecule of S) and continue plus-strand synthesis, making a tandemly duplicated plus strand. In both deletion and duplication, a replicase would synthesize the virus minus strand. Since tandem duplication of L-, M-, and S1-sized DI RNAs is not observed, the presence of two molecules of a dsRNA in one particle may be a prerequisite for tandem duplication. Two molecules of L (4.5 kb)-, M (1.8 kb)-, or S1-sized DI RNAs (1.5 kb) may not be packaged easily, whereas two molecules of S3-sized DI RNAs (0.75 kb) may.

The appearance of novel T_1 oligonucleotides in the S dsRNA's and the disappearance of M T_1 oligonucleotides originally present are indicative of the accumulation of sequence variants of the DI RNAs. All of the DI RNAs examined (including S14) are heterogeneous at the 3′ ends (Brennan and Bruenn, unpublished data), in support of this proposition. Similar changes have been observed in DI RNAs of vesicular stomatitis virus (13). Constant sequence change is only to be expected in the absence of selective pressure, and the S RNAs do not code for any proteins necessary for maintenance of the virus.

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