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NOTES

Heteroduplex Analysis of the RNA of Clone 3 Moloney Murine Sarcoma Virus

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Heteroduplex analysis of the RNA isolated from purified virions of clone 3 Moloney murine sarcoma virus (M-MSV) hybridized to cDNA's from Moloney murine leukemia virus (M-MLV) and clone 124 M-MSV shows that the main physical component of clone 3 RNA is missing all or most of the 1.5-kilobase (kb) clone 124 M-MSV specific sequence denoted β_S (S. Hu et al. Cell **10**:469-477, 1977). This sequence is either deleted in clone 3 RNA or substituted by a very short (0.3-kilobase) sequence. In other respects, clone 3 and clone 124 RNAs show the same heteroduplex structure relative to M-MLV. Since β_S is believed to contain the *src* gene(s) of clone 124 RNA, this result leaves as an unresolved question the nature of the src gene(s) of the clone 3 M-MSV RNA complex.

It is of interest to compare the sequences present in a mammalian sarcoma virus with those in its parental leukemia virus to identify those regions of the sarcoma genome which could contain the *src* genes and those sequence changes which could be responsible for the replication defect(s) of the sarcoma virus.

Moloney murine sarcoma virus (M-MSV) clone 124 and M-MSV clone 3 are murine sarcoma viruses which were derived from the original Moloney MSV stock but have had quite different passage histories (2, 3, 14, 15). The molecular lengths of the main RNA monomer physical components in the two cases are 6.0 ± 0.3 kilobases (kb) and 4.8 ± 0.3 kb, respectively (12). (In the viruses as normally propagated, the 9.0-kb component of helper Moloney murine leukemia virus [M-MLV] is present in a ratio of 1:30 and 1:5, respectively [13].)

The sequence relations between clone 124 RNA and cDNA from the parental M-MLV genome have been studied previously by electron microscope heteroduplex analysis (12). One striking feature of this structure (see Fig. 2) is a large substitution loop consisting of a 2.7-kb M-MLV sequence, β_L, which is replaced in the M-MSV by the 1.5-kb sequence, β_S. The same general feature of the structure of this M-MSV has been deduced by hybridization studies (5-7). The replication defectiveness of M-MSV may be due to any or all of the nonhomology features relative to M-MLV, namely the (θ_L, θ_S) and (β_L, β_S) substitutions and the deletions in the MSVs of the MLV-specific sequences ζ_L and δ_L (see Fig. 2). The M-MSV-specific sequence β_S has been strongly implicated as containing an *src* gene. This was accomplished by preparing full-length duplex cDNA from the 6.0-kb clone 124 RNA and observing, by DNA transfection studies, that a 2-kb restriction fragment of this duplex, spanning the β_S region, will cause morphological transformation of fibroblasts in culture (1).

It was therefore of interest to study the sequence relations of the 4.8-kb clone 3 RNA to those of the clone 124 and of the M-MLV genomes. Since the clone 3 genome is 1.0 kb shorter than that of clone 124, it cannot contain all of the sequences present in the latter.

Viruses and viral RNAs were prepared as described previously (12-15). The preparation and properties of the sample of M-MLV cDNA have been described (4). cDNA from M-MSV clone 124 RNA was synthesized similarly. The fraction of molecules in different length intervals in the M-MSV clone 124 cDNA preparation was estimated by electron microscopy to be as follows: 38%, 1 to 5 kb; 38%, 5 to 7 kb; 25%, 7 to 10 kb. RNA-cDNA hybridization and labeling of polyadenylate with SV40-polybromodeoxyuridine were carried out as previously described (4). Heteroduplex molecules were either spread di-

rectly from a 60% formamide hyperphase on to a hypophase of 15% formamide or, to achieve more complete extension of single-stranded regions, first incubated in 2 M glyoxal–0.02 M potassium phosphate buffer (pH 7.0) at 37°C for 30 min and then diluted 1:20 into spreading solution (4). Glyoxal treatment under these conditions does not significantly denature duplex segments (11).

Typical electron micrographs of molecules spread with and without glyoxal pretreatment are shown in Fig. 1. The interpretation of the heteroduplex patterns is given in Fig. 2. The only difference observed between the clone 124 MSV/MLV and clone 3 MSV/MLV heteroduplexes is as follows. In the former, there is a (β_L, β_S) substitution loop, that is, the 2.7-kb MLV specific sequence, β_L, is substituted by the nonhomologous 1.54-kb β_S sequence. All or most of the β_S sequence is missing in the clone 3 MSV/MLV heteroduplex. Without glyoxal pretreatment, in 60% of the heteroduplexes, the β_L feature appears as a deletion loop; in 40% of these heteroduplexes and in all of the glyoxal-treated molecules, there is a short (0.33 ± 0.10 kb in glyoxal spreads) sequence, β_S', in the MSV opposed to β_L of MLV. Electron microscopy is not a decisive method to discriminate between the possibilities that (i) β'_S is a short region of total or partial nonhomology of clone 3 with M-MLV, and (ii) the β'_S sequence is present in both genomes and is exposed in the heteroduplexes because of a small amount of denaturation by glyoxal or the phenomenon of "peelback" at deletion loops in formamide-cytochrome *c* spreads, or both.

In all other respects, the clone 3 MSV/MLV and clone 124 MSV/MLV heteroduplexes are identical. In both cases, we observe a small substitution loop, θ_L/θ_S, close to the 3′ end, which was not observed by Hu et al. (12). In our hands, the δ_L feature appears as a deletion loop rather than as a substitution loop (12).

The clone 3 structure was confirmed by a study of clone 3 RNA/clone 124 cDNA heteroduplexes. The only nonhomology feature in these heteroduplexes (Fig. 1) consists of the clone 124 loop β_S which is opposite an apparent short β'_S clone 3 loop. In all other respects, the clone 3 and clone 124 genomes are homologous at the heteroduplex criterion.

Thus, our most striking result is that all or most of the 1.54-kb MSV-specific sequence, β_S, of clone 124 is not present in the 4.8-kb RNA component of the clone 3 M-MSV complex. The sequence β_S probably contains the *src* genome of the clone 124 complex (1). The M-MSV clone 3 complex is able to transform mouse fibroblasts (Evans and Duesberg, personal communication) in confirmation of previous results (3). What then are the transforming genes of the clone 3 complex? The *src* gene(s) of the clone 3 complex may reside in some minor RNA component, other than the 4.8-kb molecule, which has not been detected by this heteroduplex study. In this case, the main physical component of virion clone 3 RNA with the structure depicted in Fig. 2 may be an RNA molecule in which the transformation gene has been deleted. The proportion of molecules with this deletion may differ in various passages of the virus. Alternatively, the *src* gene of clone 3 may reside in the short β'_S sequence or some other subtle nonhomology of the 4.8-kb molecule relative to M-MLV. Further biological studies are needed to resolve these questions.

In view of the possibility that the *src* gene(s) are on some minor component of the clone 3 MSV complex, we searched the clone 3/MLV heteroduplex grids for rare structures. In 140 such molecules, 114 conformed to the structure in Fig. 2 and gave quantitative data in agreement with that in the legend to Fig. 2, 19 had structures that could be attributed to hybrids between intracellular 21S mRNA and cDNA (16), and 6 had structures that did not fall into any pattern. Thus, there is no clear-cut evidence for a different *src* genome present in greater than 5% amount.

In all other respects clone 3 and clone 124 are identical or very similar in sequence at the heteroduplex criterion. Both genomes are missing the same 1.1- and 1.8-kb sequences, δ_L and ξ_L, which are present in M-MLV. Relative to M-MLV, both clone 3 and clone 124 show the small substitution loop, θ_L/θ_S, about 0.25 kb in length located about 0.5 kb from the 5′ end. Any or all of these differences with M-MLV may be responsible for the defectiveness of the M-MSV. It is possible that the absence of heterodimers in cells producing both M-MLVs and either M-MSV (13) is related to the (θ_L/θ_S) nonhomology of the MSVs with the MLV.

In summary, we have characterized the predominant component of clone 3 M-MSV RNA by heteroduplex analysis. It appears to lack the β_S region and may represent an RNA species with a deletion in the transforming gene. Then it would be analogous to transformation defective (td) variants of avian sarcoma virus. If this is so, this is the first instance observed of a deletion of a transformation gene among mammalian sarcoma viruses.

After submission of this paper, we learned that D. Donoghue at the Massachusetts Institutes of Technology has examined heterodu-

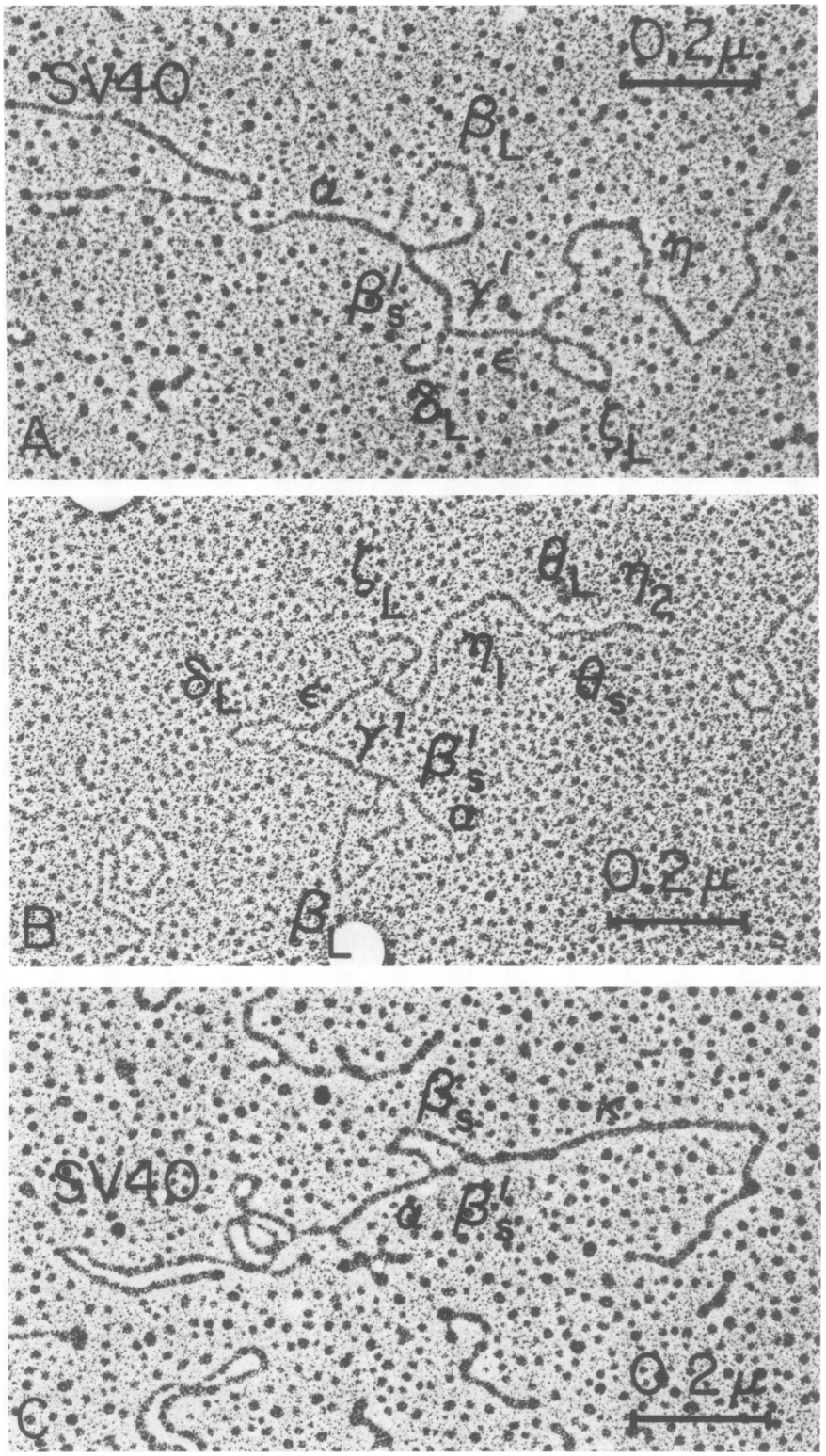

FIG. 1. *Electron micrographs of (A, B) clone 3 M-MSV/M-MLV heteroduplexes without and with glyoxal treatment, respectively, and (C) a clone 3 M-MSV/clone 124 M-MSV heteroduplex spread without glyoxal treatment.*

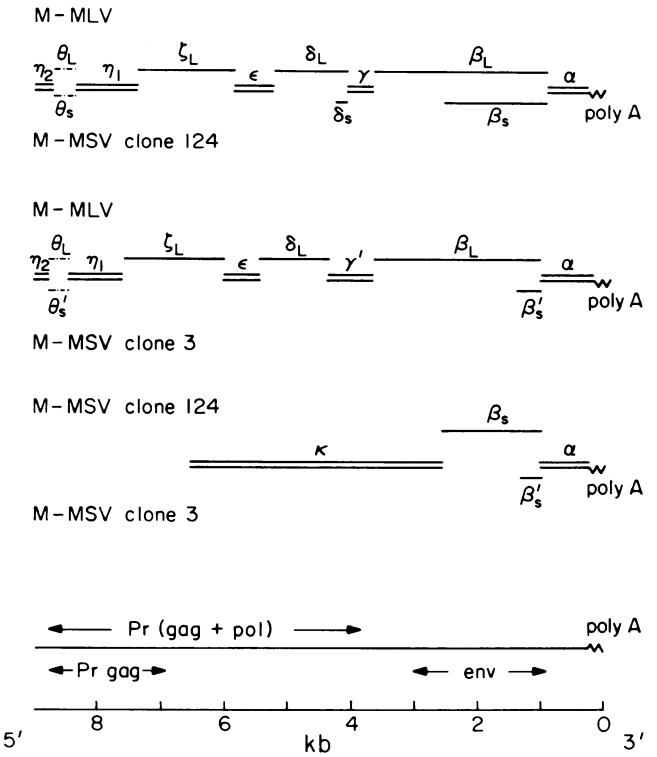

FIG. 2. *Summary of heteroduplex structures. Length measurements (in kilobases) for glyoxal-treated heteroduplexes are given below, with the numbers of molecules measured in parentheses.*

	α	β_L	β_S	β'_S	γ	δ_L	ϵ
3/MLV	0.77	2.6 ± 0.25 (20)		0.33 ± 0.10 (20)	0.67 ± 0.02 (20)	1.27 ± 0.14 (20)	0.66 ± 0.04 (20)
124/MLV	0.77	2.91 ± 0.51	1.54 ± 0.3		0.46 ± 0.10	1.26 ± 0.16	0.63 ± 0.16
3/124	0.77		1.54 ± 0.19 (27)	0.26 ± 0.10 (27)			

	ζ_L	η_1	θ_L	θ_S	η_2	κ
3/MLV	1.69 ± 0.18 (20)	1.67 ± 0.20 (16)	0.24 ± 0.08 (16)	0.24 ± 0.08 (16)	0.47 ± 0.20 (16)	
124/MLV	1.81 ± 0.23	1.61 ± 0.20 (34)	0.23 ± 0.05 (34)	0.23 ± 0.25 (34)	0.38 ± 0.10 (34)	
3/124						4.0 ± 0.18 (20)

The clone 124 MSV/MLV measurements for the features α to ζ_L are from reference 9. Note that these authors observed the δ_L features as a substitution loop, with an apposing arm $\delta_S' = 0.18 \pm 0.05$ kb. In our hands, δ_L appeared more frequently as a deletion loop. The θ_L/θ_S feature was not observed by Hu et al. (12); in our hands, it was observed frequently in either MSV/M-MLV heteroduplex and not in M-MLV homoduplex controls. The length of 0.77 kb for the α segment is from measurements by Hu et al. (12) on circular molecules. For the clone 3/MLV heteroduplex, we measured 1.00 ± 0.02 kb for linear molecules. The difference is attributed to the length of the poly(A) tail and the poly(BrdU) tail on the SV40 label on the linear heteroduplexes. A combination of the several gel electrophoretic and electron microscopic measurements by us and by Hu et al. (12) led to estimates of 4.8 ± 0.3 and 6.0 ± 0.3 kb for clone 3 and clone 124 RNAs. Donoghue et al. (8, 9) report a value of 5.2 kb for the clone 124 genome. The reason for this large discrepancy in length measurements is not known. Nevertheless, the important fact that clone 3 RNA is shorter than clone 124 by about 1 kb is clear from the several measurements by Maisel et al. (13) as well as from the data presented here.

plexes of the intracellular RNA from the NRK cell line (15), producing clone 3 MSV virions. By using methods described elsewhere (8, 9), he observes the same structure as we observe with clone 3 virion RNA. In addition, he observes at about the same frequency a second heteroduplex structure, which is like that seen by Donoghue et al. (9) with the RNA from the ml-MSV complex characterized by Fischinger et al. (10). This component has the same length as clone 3 RNA and contains the β_S segment of clone 124, but has another deletion with a length of about 1 kb, about 2 kb from the 5′ end. These observations on intracellular RNA support the hypothesis that the main virion component, whose structure we have observed, is a transformation defective deletion, and the transforming properties of the clone 3 RNA may be due to another component.

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