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Comparison of Myeloproliferative Sarcoma Virus with Moloney Murine Sarcoma Virus Variants by Nucleotide Sequencing and Heteroduplex Analysis

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The myeloproliferative sarcoma virus (MPSV) was derived by passage of Moloney sarcoma virus (Mo-MuSV) in adult mice. Mo-MuSV variants transform fibroblasts. However, MPSV also affects erythroid, myeloid, and hematopoietic stem cells. The MPSV proviral genome, two temperature-sensitive mutants derived from it, Mo-MuSV variant M1, and Moloney murine leukemia virus (Mo-MuLV) were compared by heteroduplex mapping. MPSV wild type was found to have 1 kilobase pair deleted from the *pol* gene and to contain v-*mos*-related sequences. The 3′ end of MPSV, including the oncogene-helper junctions, the v-*mos* gene, and the 3′ long terminal repeat, was sequenced and compared with sequences of Mo-MuLV, MSV-124, and the mouse oncogene c-*mos*. From these data, MPSV appears to be either closely related to the original Mo-MuSV or an independent recombinant of Mo-MuLV and c-*mos*. Five possible explanations of the altered specificity of MPSV are considered. (i) The MPSV *mos* protein has properties inherent in c-*mos* but lost by other Mo-MuSV *mos* proteins. (ii) The MPSV *mos* protein has altered characteristics due to amino acid changes. (iii) Due to a frameshift, MPSV codes for a *mos* protein truncated at the amino terminal and also a novel peptide. (iv) A second novel peptide may be encoded from the 3′ *env* region. (v) MPSV has long terminal repeats and an enhancer sequence more like Mo-MuLV than Mo-MuSV, with a consequently altered target cell specificity.

The myeloproliferative sarcoma virus (MPSV) is a member of the Moloney murine sarcoma virus (Mo-MuSV) family of replication-defective RNA tumor viruses. It is not yet clear whether all members descended from a common ancestor or whether some arose through independent recombination events. A common feature of all strains is the *mos* gene, an endogenous mouse sequence, which has been acquired by recombination with a replication-competent Moloney murine leukemia virus (Mo-MuLV) (3, 21, 31). Mo-MuSV is rendered replication defective after acquisition of the *mos* oncogene, which replaces a major part of the *env* region and also results in deletions in the *pol* gene varying in size and number among viral strains.

MPSV originated on serial transplantation in adult mice of a tumor induced in a newborn mouse by uncloned Mo-MuSV with Mo-MuLV as helper (4). The resulting MPSV-helper complex induced fibroblast transformation typical of Mo-MuSV–Mo-MuLV (24). However, unlike any other Mo-MuSV variants whose biological activity has been assayed, MPSV infection of susceptible adult mice also caused a myeloproliferative syndrome involving both myeloid and erythroid compartments of the hematopoietic system (19). These unusual pathogenic properties were shown to be due to the defective MPSV component by the induction of the myeloproliferative disease by virus rescued from cloned nonproducer MPSV cell lines (24). Nonproducer cell lines were also established for several MPSV temperature-sensitive mutants (22, 23).

The proviral genomes of MPSV and two temperature-sensitive mutants have been molecularly cloned, and restriction maps have been prepared (17). Both this and the earlier work of Pragnell et al. (29) show that, within the limits of resolution of the techniques used, MPSV has no apparent novel cellular sequences or striking rearrangements that could account for its particular effects. In this study we examined the structural organization of each MPSV clone with respect to Mo-MuLV and the Mo-MuSV variant M1 by heteroduplex analysis. This provides additional evidence for the close similarity of MPSV to the Mo-MuSV family. We also sought to analyze the evidently subtle differences between MPSV and the Mo-MuSV variant MSV-124 by means of DNA sequence analysis of the proviral genome in the region around the *onc* gene and the 3′ long terminal repeats (LTR). This was an attempt, if not to actually explain the MPSV phenotype, then to allow the framing of specific questions concerning the importance of particular features in subsequent experiments.

MATERIALS AND METHODS

Plasmids. The wild-type MPSV plasmid p18-663 and MPSV temperature-sensitive plasmids p20-159 and p19-124 have been described previously (17). Moloney MuLV clone Mov-3 was provided by R. Jaenisch, and Mo-MuSV M1 was provided by G. Vande Woude (34, 35) and subcloned into pAT153 by D. Hughes.

Sequence analysis. Cloned MPSV DNA was subcloned into M13 vectors and sequenced by the method of Sanger et al. (30). A set of random overlapping subclones covering the region from bases 1 to 2020 (see Fig. 3) was generated by recircularization of the 2-kilobase pair (Kbp) *Hin*dIII fragment of p18-663, followed by ultrasonication, end repair using the Klenow fragment of *Escherichia coli* DNA polymerase I, and size fractionation on an agarose gel. Fragments between 200 and 700 base pairs (bp) were recovered and blunt-end ligated into *Sma*I-cut, alkaline phosphatase-treated M13 mp8.

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Subclones covering bases 2021 to 2854 of p18-663 and also specific regions of p20-159 and p19-124 were generated by various combinations of restriction enzyme digestions (see restriction maps in reference 17) and ligation of chosen fragments in the desired orientation into either M13 mp8 or mp9.

Electron microscopy. Cloned plasmid DNAs were prepared by the method of Godson and Vapnek (13) and purified by isopycnic centrifugation on cesium chloride-ethidium bromide gradients. DNA samples were prepared for electron microscopy by a modification of the method of Davis et al. (5). Recombinant DNA samples were digested with *Eco*RI, mixed in equimolar ratios, and denatured by heating at 70°C for 2 min in 100 mM Tris (pH 8.5)–10 mM EDTA–50% formamide. Samples were allowed to reanneal at 37°C for a minimum of 30 min. DNA was spread from 20 mM Tris (pH 8.5)–2 mM EDTA–40% formamide–100 μg of cytochrome *c* per ml onto a 10 mM Tris (pH 8.5)–1 mM EDTA–10% formamide hypophase. Grids were stained with uranyl acetate and rotary shadowed with platinum-palladium. Single- and double-stranded circular pAT153 molecules were added to each experiment to provide internal size standards.

RESULTS

Heteroduplex analysis of MPSV. The cloned MPSV provirus was hybridized to Mo-MuLV or M1 proviral DNA, and the resulting heteroduplex molecules were analyzed (Fig. 1b and d). The data revealed that MPSV has conserved 2.6 kbp at the 5′ end of the genome, which would be expected to contain most, if not all, of the *gag* gene. However, compared with Mo-MuLV, MPSV has 1.0 kbp deleted from the *pol* gene. The 5′ end of this deletion maps close to the *gag-pol* junction, but the loss of some 3′ *gag* sequences cannot be excluded because of the size (±0.26 kbp) of the standard deviation of the measurement. The observation that M1-MPSV heteroduplexes contain a simple deletion loop (Fig. 1d) shows that the 5′ end of the MPSV deletion must be close to that of M1. If it were more than about 50 bp to the 5′ side, a recognizable substitution loop would be formed. Clarification of this point awaits sequencing of this region of MPSV.

Compared with Mo-MuLV, MPSV has a substitution of 1.3 kbp in the *env* gene. This hybridized to the v-*mos* region of M1 (Fig. 1d), showing that MPSV contains the v-*mos* gene in a similar position and in the same orientation as other Mo-MuSV variants. The LTR sequences of all three proviruses appear to be broadly homologous, although heteroduplex mapping would not be expected to detect deletions or substitutions of less than 50 to 100 bp in this region or elsewhere in the genome.

Heteroduplex analysis of temperature-sensitive mutants. The temperature-sensitive mutants ts 124 and ts 159 were compared with Mo-MuLV, M1, and MPSV and with each other. The heteroduplexes formed by ts 124 were identical to those of MPSV (data not shown). Presumably, the cause of temperature sensitivity in this virus is a mutation which is too small to be detected by heteroduplex mapping. Comparisons of the ts 159 provirus with Mo-MuLV or M1-MSV showed that it contained a v-*mos* substitution in the *env* region, apparently identical to MPSV and other MuSV variants (Fig. 1c and e). Heteroduplexes of ts 159 to MPSV (Fig. 1f) showed that, in addition to the 1.0-kbp deletion present in MPSV, ts 159 contained a further deletion of 1.5 kbp in the *pol* gene. The 300 bp of *pos* sequence between the two deletions, which is present in MPSV and ts 159, is not maintained in M1 (Fig. 1c). The 1.5-kbp deletion was not present in the original temperature-sensitive mutant cell line

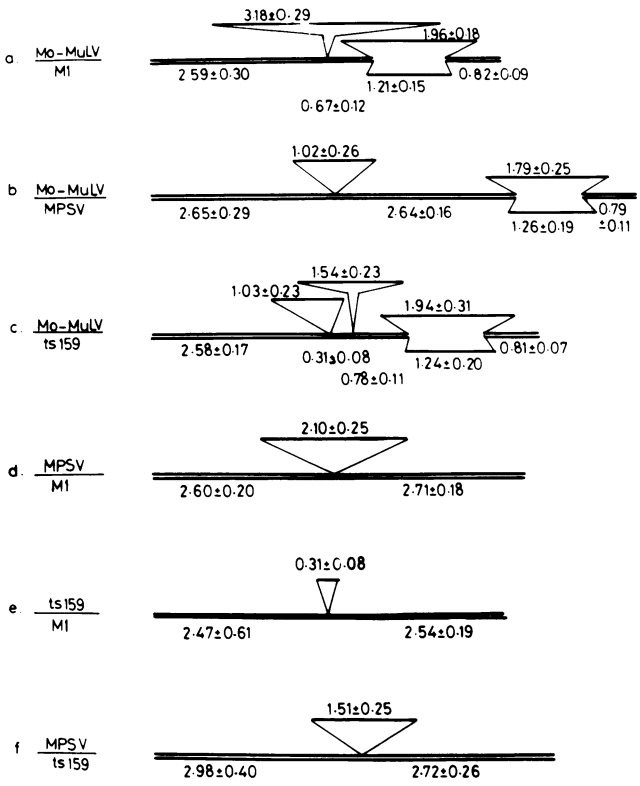

FIG. 1. Summary of heteroduplex analyses. A minimum of 20 molecules from each experiment were measured, and sizes were calculated using internal standards as described in the text. All molecules are drawn to scale, with the flanking sequences omitted, and the mean size and standard deviation in kbp of each component are shown.

(17), so it cannot be the primary cause of the observed temperature sensitivity, but probably lies in a region of the defective genome which is readily deleted during passaging of cells. It may be concluded that these two temperature-sensitive mutants are clearly closely homologous to MPSV, and their acquisition of temperature sensitivity has not involved gross sequence changes.

Sequence data. The MPSV proviral genome was sequenced from the *Hin*dIII site 895 bp upstream of the Mo-MuLV *env-mos* junction to the end of the 3′ LTR. The first base shown in Fig. 2 corresponds to base 4893 of the Mo-MuLV sequence (37). This sequence has been compared with sequences of MSV-124, Mo-MuLV, and c-*mos* published previously (32, 33) but slightly updated and collected (37). The predicted MPSV *mos* amino acid sequence is given in Fig. 3a and compared with those of c-*mos* and MSV-124 *mos*.

MPSV 5′ *env-mos* junction. The position of the *env-mos* junction is identical in the Mo-MuSV variants MSV-124, HT1, M1, and Gazdar (10). All of these variants have potential initiation sites for translation of the *mos* message in the *env* sequence, reading through into the *mos* region. MPSV has one less cytosine residue at this junction point, which may be regarded either as a different junction or as a subsequent deletion. The missing C would not seem to be an artifact of molecular cloning, as it was not found in any of the three independently isolated MPSV clones (i.e., MPSV wild type and two temperature-sensitive mutants) which were sequenced over this region. The consequence of this is that

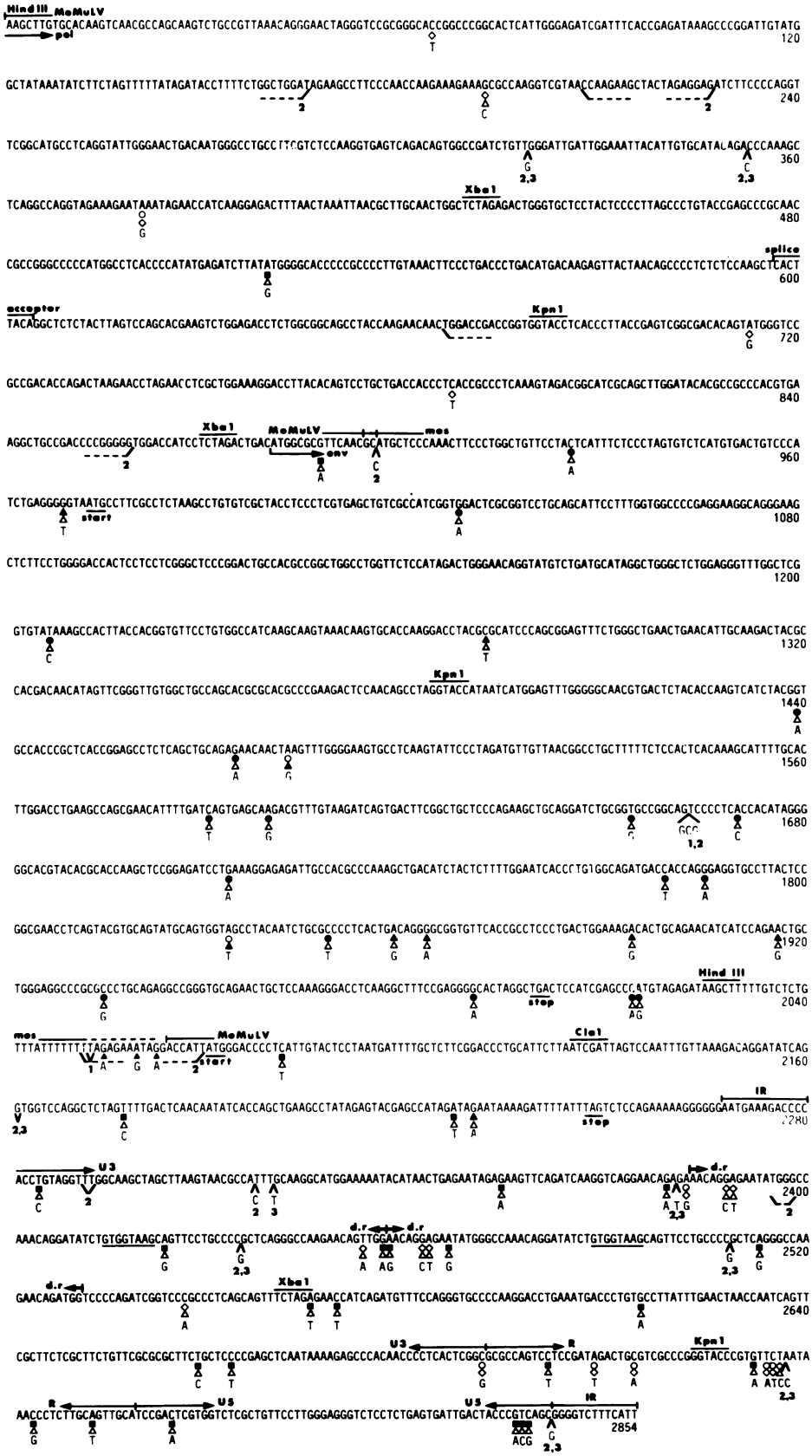

FIG. 2. Nucleotide sequence of MPSV from 895 bp upstream of the *mos* gene, including differences from Mo-MuLV, MSV-124, and c-*mos* (37). Base 1 corresponds to base 4893 of Mo-MuLV. Nucleotides 2853 and 2854 were absent from the proviral form but were inferred as homologous to nucleotides 2268 and 2269. Symbols: ●, nucleotide is the same as c-*mos*; ○, nucleotide is the same as MSV-124; ■, nucleotide is the same as Mo-MuLV; ▲, nucleotide differs from c-*mos*; △, nucleotide differs from MSV-124; ◇, nucleotide differs from Mo-MuLV; ∧, deletion from MPSV as compared with another sequence (indicated by the number); ∨ ⌊_____⌋, region present in MPSV but absent in another sequence (indicated by the number). 1, c-*mos*; 2, MSV-124; 3, Mo-MuLV. d.r., Direct repeats.

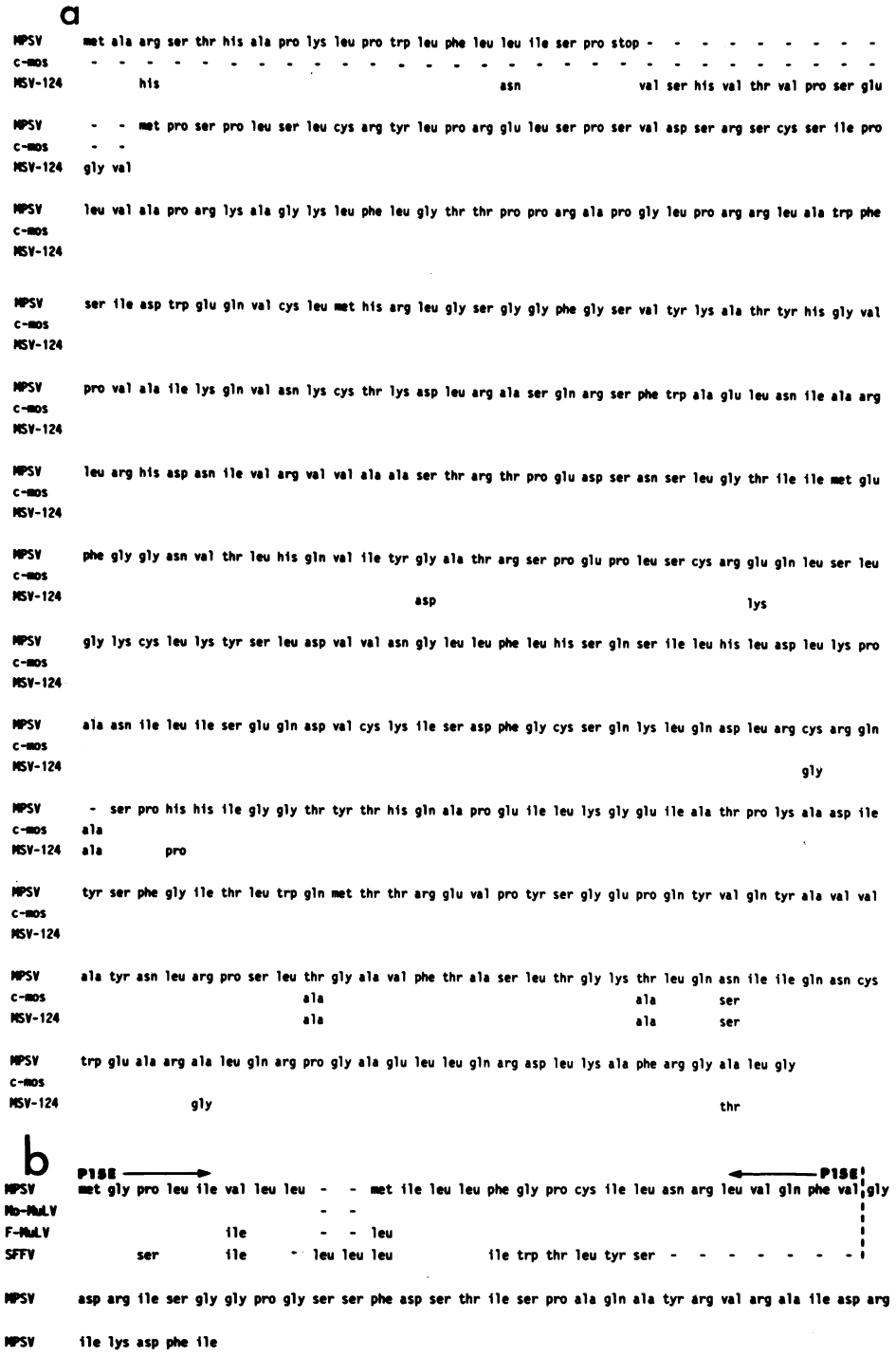

FIG. 3. (a) Predicted amino acid sequence of the MPSV *mos* coding region corresponding to bases 1000 to 1999 of Fig. 2, with comparisons made with c-*mos* and Mo-MuSV *mos*. (b) Predicted amino acid sequence of the MPSV novel peptide, encoded by the 3′ *env* region, corresponding to bases 2070 to 2245 of Fig. 2, with comparisons made with Mo-MuLV, Friend MuLV, and SFFV in the section derived from the p15E region.

any *env-mos* product initiated at the AUG in the *env* region will suffer a frameshift which establishes the 20th codon as an amber terminator. This short open reading frame could encode a 19-amino acid peptide different from, but similar in charge and number of proline residues to, the amino terminal of *env-mos*. The next AUG, marked start in Fig. 2, is 92 bases after the first, reopens the main reading frame in phase, and could be expected to initiate the translation of a *mos* protein lacking 31 amino acids at the amino terminal.

MPSV *mos*. The *mos* sequence is broadly similar to c-*mos* and MSV-124 *mos*. Of the 21 single base differences between the two viral *mos* genes and c-*mos* within the MPSV major

coding region, MPSV is identical to c-*mos* in 14 cases, the same as MSV-124 in 2 cases, and differs from both in 5 cases. Furthermore, on examination of the amino acid sequence (Fig. 3a), it is apparent that where MPSV resembles MSV-124, the change is silent, but in 6 of the 14 instances in which MPSV and c-*mos* are identical, the amino acid sequence is altered in MSV-124. These latter six include the loss of a cysteine residue present in c-*mos* and the replacement of a histidine by a proline, both of which could conceivably alter the final protein conformation.

Four of the six changes unique to MPSV alter amino acids, two replace alanine with threonine, and one replaces serine with asparagine, with no net alteration of charge. The fourth is a product of a triplet deletion and removes an alanine residue. The importance of these novel attributes of the MPSV *mos* is difficult to assess without knowledge of the functional domains of the protein.

MPSV 3′ *mos-env* junction. The precise position of the *mos-env* junction is ambiguous from the sequence data. Perfect homology with c-*mos* stops at base 2051 (Fig. 2), which is precisely the same junction point as MSV-124. However, homology to Mo-MuLV does not commence until base 2063. The intervening 12 nucleotides have a 75% homology with c-*mos* and no appreciable homology with either MSV-124 or Mo-MuLV.

MPSV *env* sequences. In the remaining *env* sequences 3′ to the *mos-env* junction there are five differences between MPSV and MSV-124; in three of these MPSV resembles Mo-MuLV; the other two, including a singe base insertion, are new to MPSV. This again suggests a similarity with the original helper virus rather than with MSV-124.

MPSV 3′ LTR. Once again the LTR region bears a closer resemblance to Mo-MuLV than to MSV-124. MPSV shows 13 bases unlike Mo-MuLV, of which 6 are like MSV-124, and 28 bases are unlike MSV-124, of which 21 are like Mo-MuLV. MPSV carries two separate stretches of 2 and 5 bases, which are present in Mo-MuLV but deleted from MSV-124, and lacks a single base insertion present in MSV-124. There are five point deletions unique to MPSV and one held in common with MSV-124.

A 74-base sequence is present as two contiguous direct repeats (Fig. 2) which differ by a single base. The position of these repeats within the LTR is identical to the 75-bp repeats of Mo-MuLV found between bases 7933 and 8082 in the published sequence (37). The length of MPSV repeats is reduced to 74 bp by a matched pair of single base deletions (by bases 2434 and 2508 [Fig. 2]). It may be significant that in all but a single base change, where MPSV has diverged from Mo-MuLV, the differences occur as pairs identical between each repeat (bases 2488 and 2462, 2489 and 2463, and the deletions mentioned above). This would seem to indicate some presumably functional constraint on the integrity of the sequences in MPSV.

In contrast, MSV-124 has diverged considerably from Mo-MuLV in this region and has lost homology between each repeat in 16 bases at the 5′ end and the last base at the 3′ end. This separates each repeat by 18 bases and reduces their lengths to 58 bp (37).

DISCUSSION

The heteroduplex analyses presented here show that MPSV and two of its temperature-sensitive mutants are closely related to Mo-MuLV and to those Mo-MuSV variants for which there are published data. The entire MPSV genome appears to be derived from Mo-MuLV or c-*mos* (29). Differences in the pathogenesis through differing target cell specificities of MPSV and Mo-MuSV cannot be explained by novel host-derived sequences expressed in MPSV. Compared with Mo-MuLV, we have found that MPSV contains a 1.0-kbp deletion in the *pol* gene, in agreement with the data of Kollek et al. (17). All other Mo-MuSV variants show *pol* deletions, but MPSV has the smallest so far described. The structure of MPSV ts 159 suggests that *pol* deletions arise readily during the passaging of virus-infected cell lines. This effect may be responsible for the many Mo-MuSV variants that differ only in this respect. This most probably reflects the unimportance of the region to the defective virus. Canaani et al. (3) reported a variant clone 14, which deleted the entire *pol* gene and the 3′ end of the *gag* gene, thereby setting a precedent for the idea that the 3′ end of the *gag* gene is also nonessential. No conclusions as to the relationship between the Mo-MuSV variants and MPSV can be drawn with any confidence from these differences alone. Partial nucleotide sequencing of the most interesting sections of the genome has allowed more detailed analysis.

Sequence data covering the *mos*-helper junctions of MSV-124, HT1, M1, and Gazdar have been used as the basis for a proposed interrelationship among Mo-MuSV variants (12). Whereas the 3′ *mos-env* junction is quite variable between these strains, the 5′ *env-mos* junction has the same position throughout. This may suggest either a recombination "hot spot," a functional requirement for the 5′ *env* fragment, or may simply be due to the common origin of the family.

Four nucleotides before the *env-mos* 5′ junction point of MPSV and Mo-MuSV there is a sequence, TTCA, which is common to both Mo-MuLV and c-*mos*. In the simian sarcoma virus a region of six nucleotides has been reported (14) as similarly showing homology between helper sequences 5′ to the helper-oncogene junction and cellular sequences upstream from those acquired by the virus and is postulated as having played a role in the initial recombination between the virus and the host genome. The 4-base region in the Mo-MuSV variants and MPSV could provide a hot spot, but as MPSV may have formed a 5′ junction differing by one base (Fig. 2), possibly junctions would not seem absolutely restricted to a single point.

Unfortunately, comparison of the MPSV junction data with the other junction sequences does not clarify the relationship of MPSV to the Mo-MuSV variants. The 5′ junction is possibly new, and the 3′ junction includes 12 bases originally from c-*mos* which are not found in MSV-124. However, these two points, considered together with the close homology of MPSV with both Mo-MuLV and c-*mos* and the fact that MPSV has a minimally deleted *pol* gene (Fig. 1b), seem to indicate that MPSV represents either an independent recombinant between Mo-MuLV and c-*mos* or some form close to the original Mo-MuSV recombinant, although it is possible that the small *pol* deletion is due to restoration of lost sequences by a secondary recombination with Mo-MuLV. Examination of the base substitutions throughout the region sequenced shows no particular relatedness of MPSV to MSV-124 over that expected by coincidence alone, which could indicate an entirely independent origin for MPSV. The present sequence data alone are not sufficient to distinguish among the various possibilities. This matter could possibly be resolved by analysis of the nucleotide sequence of Mo-MuSV HT1, which has been proposed as closely related to the putative common ancestor of the Mo-MuSV family (10, 11).

As the MPSV *mos* amino acid sequence resembles c-*mos* more closely than does the MSV-124 *mos* sequence, it could

be argued that the transformation of hematopoietic stem cells is a property of the unaltered c-*mos* protein and has been lost through alteration by the Mo-MuSV family. If it transpires that HT1 *mos* is identical to c-*mos*, then the similarity of MPSV *mos* to c-*mos* could be ruled out as a possible cause of MPSV action. Any differences that do exist would, on comparison with MPSV, narrow down the number of potentially crucial amino acid changes (Fig. 3a), which could give rise to novel attributes of the MPSV *mos* protein.

More direct tests of the properties of c-*mos* are in progress at this time and develop the work of Blair et al. (2). These involve the placement of c-*mos* between two chosen LTRs, using the constructs to productively transfect fibroblasts, and then investigating possible erythroleukemic effects of the recovered viral preparations in vivo.

The 5′ *env-mos* junction of MPSV sets it apart from all the other Mo-MuSV variants. If the constancy of the junction in the Mo-MuSV family represents the preservation of a function, then the alteration in MPSV could be a cause of new characteristics. The consequences of the frameshift originating at the MPSV junction do include changes in the proteins encoded by *mos*; thus, phenotypic differences are possible.

Papkoff et al. (28) showed that the overlapping series of proteins of molecular weights 37,000, 33,000, 24,000, and 18,000 previously found in in vitro translates of MSV-124 and Gazdar genomic and subgenomic RNA (25, 26) are produced by initiation at the different AUG codons of the region and share common carboxyl termini. p37, which has an amino terminus proposed as corresponding to the AUG within the 5′ *env* fragment, has been detected in MSV-124-transformed NIH 3T3 cells (27) and is strongly implicated as the transforming protein. However, there is a lack of direct biochemical evidence regarding the splicing of the *mos* RNA transcript in vivo. The splice acceptor site marked in Fig. 2 was deduced as a consensus sequence by Shinnick et al. (32), and the proposed pattern of splicing assumes that the *env* AUG is available for initiation in the final message.

Accepting this splicing pattern, our data predict that MPSV is unable to produce p37 from the *env* initiation site and could be expected to produce p34 from the first internal AUG. Blair et al. (D. G. Blair, T. G. Wood, A. M. Woodworth, M. L. McGready, M. K. Oskarsson, F. Propst, M. Tainsky, C. S. Cooper, R. Watson, B. M. Baroudy, and G. F. Vande Woude, *The Cancer Cell*, in press) have revised the upstream c-*mos* sequence of Van Beveren et al. (33) and state that this same AUG is the first to be followed by a long open reading frame. The ability of MPSV and c-*mos* in LTR constructs to transform fibroblasts implies that 31 amino acids at the terminal of p37 are unnecessary for this particular function. Whether p34 and p37 have different properties remains to be tested. The failure to detect p34 in Mo-MuSV transformed fibroblasts (27) might suggest that its expression is repressed when translation of p37 is possible.

A further consequence of the frameshift at the 5′ junction is the predicted presence of a novel peptide initiated at the *env* AUG. This is detailed in Fig. 3a. However, it is doubtful whether such a short peptide could be synthesized in vivo.

The translation of a second peptide novel to MPSV may occur due to an insertion at base 2161 (Fig. 2). An open reading frame exists within the helper region from which MPSV could encode a peptide with an amino end comprising 30 amino acids from p15E, linked to 29 amino acids derived from, but in a different reading frame to, the R region (32). This chimeric p15E-MPSV peptide would have a high incidence of basic amino acids and a cysteine residue available for disulfide bonding. It may be noted that the part of this peptide encoded by the in-frame p15E region is largely that of a hydrophobic stretch of amino acids (underlined in Fig. 3b). This has been suggested by Wolff et al. (38) as possibly important to the function of the SFFV gp55, the carboxyl terminus of which is derived from p15E. If expressed, this peptide might prove to be a common factor between MPSV and other erythroleukemia viruses. Most of the gp70 and p15E sequences are deleted in all of the Mo-MuSV variants, and the hypothetical R protein, which could not be translated by MPSV, has not been detected in any of the Mo-MuSV variants.

It is possible that either of the two predicted novel MPSV peptides may exert independent effects or interact with the *mos* protein. Antisera against *mos* and synthetic peptides corresponding to those predicted may be used to determine the pattern of expression, including the size of the *mos* protein, in MPSV-infected cells.

There are several indirect indications that the LTRs may be the source of MPSV activity. Anderson and Scolnick (1) found that acute erythroproliferative disease could be induced in mice by a recombinant virus constructed from the avian Rous sarcoma virus *src* gene, when inserted into the Mo-MuLV *env* region in a manner analogous to MPSV. Rous sarcoma virus, which usually transforms chicken fibroblasts, replicates very poorly in murine cells. Such a dramatic change in the species specificity and pathological effects of the oncogene brought about by the new viral context suggests that, in so far as target cell specificity is concerned, the oncogene is less important than generally imagined, in line with ideas proposed by Duesberg (12).

In the case of the Mo-MuLV–*src* recombinant, no helper function can be excluded with certainty from having a possible effect on the type of cell transformed. However, the LTRs seem particularly implicated, as the *gag* and *pol* regions are not required for transformation by Moloney sarcoma viruses, an idea supported by the lack of consistent differences in *gag* and *pol* between MPSV and the Mo-MuSV variants. Furthermore, it has been shown (2, 9, 39) that linkage of an isolated Mo-MuSV LTR, containing at minimum the U3 region, to c-*mos* or Mo-MuSV *mos* is necessary and sufficient, in the absence of other viral regions, to render a plasmid construct capable of fibroblast transformation. Differences in the LTR direct repeats of murine leukemia viruses have also been suggested as the source of the different tissue tropisms of various strains (6).

Mo-MuSV LTR direct repeats have been used to functionally replace the simian virus 40 direct repeats as enhancers of early viral transcription (20). In view of the emerging importance of enhancers as regulators of transcription (16), it is possible that this area in particular contributes to the specific activity of MPSV.

The differential activity of isolated viral enhancers in various cellular environments has been demonstrated, for example, in the host-specific modulation of expression of genes adjacent to simian virus 40, polyoma, and Mo-MuSV enhancers by transfected mouse and monkey cells (7, 18). The importance of the nucleotide sequence of the enhancer in directing the transformation of a particular cell type is also established from analysis of those polyoma mutants capable of transforming embryonal carcinoma cells (15), although the polyoma enhancer is not of the direct repeat type. However, no effects on direct repeat enhancer activity or specificity due to alterations outside the "core sequence" (underlined in Fig. 3) (36) have yet been demonstrated. Indeed, Mo-MuSV can tolerate large alterations, including the loss of a complete repeat, without loss of fibroblast transforming

activity in vitro (R. Narayanan, personal communication). This seems hard to reconcile with the apparent maintenance of direct homology between the MPSV repeats under the pressure of mutations, unless fundamentally different processes are involved during the transformation of fibroblasts in vitro and of hemopoietic cells of fibroblasts (induction of sarcomas) in the whole animal.

If a regulatory function is ascribed to the LTR direct repeats which entails recognition and modulation by cellular factors, it is not difficult to imagine that interaction being significantly affected by the degree of sequence disruption that has occurred during the divergence of MSV-124 from Mo-MuLV. However, too many conclusions should not be drawn about the significance of the enhancers of MPSV and the Mo-MuSV variants from comparisons made with MSV-124 alone. Sequencing of the Mo-MuSV M1 LTR (8) has shown that it has direct repeats of 73 and 72 bp that are only a little changed from those of Mo-MuLV. In M1 the original contiguous 75 base repeats have diverged from Mo-MuLV and lost homology with each other by 3 base substitutions and a single base deletion, whereas MPSV maintains homology between repeats in all but a single base. Interestingly, M1 also has the same matched pair of deletions found in MPSV, which would argue against any MPSV-specific function for them. M1 displays no detectable erythroleukemic effects (22) and therefore may provide a test for the hypothetical role of the enhancer in determining MPSV specificity. Given a positive answer to this, M1 may further pinpoint particular features of the direct repeats which are important.

To summarize, the particular effects of MPSV seem explicable in five possible ways. (i) Myeloproliferative disease and fibroblast transformation are the natural properties of the mouse c-*mos* oncogene carried, unaltered in critical areas, by MPSV. (ii) The MPSV *mos* protein has undergone slight novel changes in its amino acid sequene which alter its structure sufficiently to affect the range of cell types transformed. (iii) Due to a frameshift at the 5′ *env-mos* junction, MPSV codes for a truncated *mos* protein and a novel peptide. (iv) A second frameshift in the 3′ *env* region allows the encoding of another novel peptide. (v) The type of disease induced by the oncogene is not a function of the oncogene itself but rather of the different cell type(s) most compatible with the particular enhancer structure of the infecting virus and therefore the most readily transformed.

These points are not mutually exclusive, complete explanations, but rather a series of propositions arising from the sequence analysis which are verifiable by future experimentation.

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LITERATURE CITED

1. **Anderson, S. M., and E. M. Scolnick.** 1983. Construction and isolation of a transforming murine retrovirus containing the *src* gene of Rous sarcoma virus. J. Virol. **46**:594–605.
2. **Blair, D. G., M. Oskarsson, T. G. Wood, W. L. McClements, P. J. Fischinger, and G. G. Vande Woude.** 1981. Activation of the transforming potential of a normal cell sequence: a molecular model for oncogenesis. Science **212**:941–943.
3. **Canaani, E., K. C. Robbins, and S. A. Aaronson.** 1979. The transforming gene of Moloney murine sarcoma virus. Nature (London) **282**:378–383.
4. **Chirigos, M. A., D. Scott, W. Turner, and K. Perk.** 1968. Biological, pathological and physical characterization of a possible variant of a murine sarcoma virus (Moloney). Int. J. Cancer **3**:223–237.
5. **Davis, R. W., M. Simon, and N. Davidson.** 1971. Electron microscope heteroduplex methods for mapping regions of base sequence homology in nucleic acids. Methods Enzymol. **21**:413–428.
6. **Des Groseillers, L., E. Rassort, and P. Jolicoeur.** 1983. Thymotropism of murine leukemia virus is conferred by its long terminal repeat. Proc. Natl. Acad. Sci. U.S.A. **80**:4203–4207.
7. De Villiers, J., L. Olson, C. Tyndall, and W. Schaffner. 1982. Transcriptional 'enhancers' from SV40 and polyoma virus show a cell type preference. Nucleic Acids Res. **10**:7965–7976.
8. **Dhar, R., W. L. McClements, L. W. Enquist, and G. F. Vande Woude.** 1980. Nucleotide sequences of integrated Moloney sarcoma provirus long terminal repeats and their host and viral junctions. Proc. Natl. Acad. Sci. U.S.A. **77**:3937–3941.
9. **Donoghue, D. J.** 1982. Demonstration of biological activity and nucleotide sequence of an in vitro synthesized clone of the Moloney murine sarcoma virus *mos* gene. J. Virol. **42**:538–546.
10. **Donoghue, D. J., and T. Hunter.** 1983. Recombinational junctions of variants of Moloney murine sarcoma virus: generation and divergence of a mammalian transforming gene. J. Virol. **45**:607–617.
11. **Donoghue, D. J., P. A. Sharp, and R. A. Weinberg.** 1979. Comparative study of different isolates of murine sarcoma virus. J. Virol. **32**:1015–1027.
12. **Duesberg, P. H.** 1980. Transforming genes of retroviruses. Cold Spring Harbor Symp. Quant. Biol. **44**:13–29.
13. **Godson, G. N., and D. Vapnek.** 1973. A simple method of preparing large amounts of ϕX 174 RFI supercoiled DNA. Biochim. Biophys. Acta **299**:516–523.
14. **Josephs, S. F., R. Della Favera, E. P. Gelmann, R. C. Gallo, and F. Wong-Staal.** 1983. 5′ Viral and human cellular sequences corresponding to the transforming gene of simian sarcoma virus. Science **219**:503–505.
15. **Katinka, M., M. Vasseur, N. Montreau, M. Yaniv, and D. Blangy.** 1981. Polyoma DNA sequences involved in control of viral gene expression in murine embryonal carcinoma cells. Nature (London) **290**:720–722.
16. **Khoury, G., and P. Gruss.** 1983. Enhancer elements. Cell **33**:313–314.
17. **Kollek, R., C. Stocking, F. Smadja-Joffe, and W. Ostertag.** 1984. Molecular cloning and characterization of a leukemia-inducing myeloproliferative sarcoma virus and two of its temperature-sensitive mutants. J. Virol. **50**:717–724.
18. **Laimins, L. A., G. Khoury, C. Gorman, B. Howard, and P. Gruss.** 1982. Host-specific activation of transcription by tandem repeats from simian virus 40 and Moloney murine sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **79**:6453–6457.
19. **Le Bousse-Kerdiles, M. C., F. Smadja-Joffe, B. Klein, B. Caillou, and C. Jasmin.** 1980. Study of a virus-induced myeloproliferative syndrome associated with tumor formation in mice. Eur. J. Cancer **16**:43–51.
20. **Levinson, B., G. Khoury, G. F. Vande Woude, and P. Gruss.** 1982. Activation of SV40 genome by 72 base pair tandem repeats of Moloney sarcoma virus. Nature (London) **295**:568–572.
21. **Moloney, J. B.** 1966. A virus induced rhabdomyosarcoma of mice. Natl. Cancer Inst. Monogr. **22**:139–142.
22. Ostertag, W., M. Freshney, K. Vehmeyer, C. Jasmin, and G. Rutter. 1984. Action of temperature-sensitive mutants of myeloproliferative sarcoma virus suggests that firbroblast-transforming and hematopoietic transforming viral properties are related. J. Virol. **49**:253–261.
23. **Ostertag, W., I. B. Pragnell, A. Fusco, D. Hughes, M. Freshney, B. Klein, C. Jasmin, J. Bilello, G. Warnecke, and K. Vehmeyer.** 1982. Studies on the biology and genetics of murine erythroleukemia viruses, p. 401–416. *In* R. F. Revoltella (ed.), Expression of differential functions in cancer cells. Raven Press, New York.

24. **Ostertag, W., K. Vehmeyer, B. Fagg, I. B. Pragnell, W. Paetz, M. C. Le Bousse, F. Smadja-Joffe, B. Klein, C. Jasmin, and H. Eisen.** 1980. Myeloproliferative virus, a cloned murine sarcoma virus with spleen focus forming properties in adult mice. J. Virol. **33**:573–582.
25. **Papkoff, J., and T. Hunter.** 1982. Identification of proteins encoded by the Gazdar murine sarcoma virus genome by in viro translation and comparison with Moloney murine sarcoma virus 124. J. Virol. **43**:533–543.
26. **Papkoff, J., T. Hunter, and K. Beemon.** 1980. In vitro translation of virion RNA from Moloney murine sarcoma virus. Virology **101**:91–103.
27. **Papkoff, J., E. A. Nigg, and T. Hunter.** 1983. The transforming protein of Moloney murine sarcoma virus is a soluble cytoplasmic protein. Cell **33**:161–172.
28. **Papkoff, J., I. Verma, and T. Hunter.** 1982. Detection of a transforming gene product in cells transformed by Moloney murine sarcoma virus. Cell **29**:417–426.
29. **Pragnell, I. B., A. Fusco, C. Arbuthnott, F. Smadja-Joeffe, B. Klein, C. Jasmin, and W. Ostertag.** 1981. Analysis of the myeloproliferative sarcoma virus genome: limited changes in the prototype lead to altered target cell specificity. J. Virol. **38**:952–957.
30. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A. **74**:5463–5467.
31. **Scolnick, E. M., R. A. Howk, A. Anisowicz, P. T. Peebles, C. D. Scher, and W. P. Parks.** 1975. Separation of sarcoma virus-specific and leukemia virus-specific genetic sequences of Molony sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **72**:4650–4654.
32. **Shinnick, T. M., R. A. Lerner, and J. G. Sutcliffe.** 1981. Nucleotide sequence of Moloney murine leukaemia virus. Nature (London) **293**:543–548.
33. **Van Beveren, C., J. A. Galleshaw, V. Jonas, A. J. M. Berns, R. F. Doolittle, D. J. Donoghue, and I. M. Verma.** 1981. Nucleotide sequence and formation of the transforming gene of a mouse sarcoma virus. Nature (London) **289**:258–262.
34. **Vande Woude, G. F., M. Oskarsson, L. W. Enquist, S. Nomura, M. Sullivan, and P. J. Fischinger.** 1979. Cloning of integrated Moloney sarcoma proviral DNA sequences in bacteriophage lambda. Proc. Natl. Acad. Sci. U.S.A. **76**:4464–4468.
35. **Vande Woude, G. F., M. Oskarsson, W. L. McClements, L. W. Enquist, D. G. Blair, P. J. Fischinger, J. V. Maizel, and M. Sullivan.** 1979. Characterization of integrated Moloney sarcoma proviruses and flanking host sequences cloned in bacteriophage lambda. Cold Spring Harbor Symp. Quant. Biol. **44**:735–745.
36. **Weiher, H., M. König, and P. Gruss.** 1983. Multiple point mutations affecting the simian virus 40 enhancer. Science **219**:626–631.
37. **Weiss, R., N. Teich, H. Varmus, and J. Coffin (ed.).** 1982. Three retroviral genomes and a cellular *onc* gene, p. 1349–1375. *In* Molecular biology of tumor viruses, 2nd ed., RNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
38. **Wolff, L., E. Scolnick, and S. Ruscetti.** 1983. Envelope gene of the Friend spleen focus-forming virus: deletion and insertions in 3′ gp70/p15E-encoding region have resulted in unique features in the primary structure of its protein product. Proc. Natl. Acad. Sci. U.S.A. **80**:4718–4722.
39. **Wood, T. G., M. L. McGeady, D. G. Blair, and G. F. Vande Woude.** 1983. Long terminal repeat enhancement of v-*mos* transforming activity: identification of essential regions. J. Virol. **46**:726–736.