Polyomavirus Growth and Persistence in Friend Erythroleukemic Cells

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Infection of Friend erythroleukemic (FL) cells by polyomavirus (Py) invariably results in the selection of persistently infected FL-Py cell lines and clones. Anti-Py serum treatment of FL-Py lines and clones leads to the loss of Py genome and consequent cell cure. Conversely, cure has not been obtained in FL-PytsA cell lines (isolated after infection by a Py thermosensitive early mutant) and their derivative clones cultivated for a long time at nonpermissive temperature (39°C), where viral large-T protein is inactive. Rescue of viral particles has always been obtained after shifting cells to 32°C. Integrated viral genomes were detected by blot hybridization in an FL-PytsA clone at 39°C. Long-term observation of FL-Py cell lines and their derivative clones reveals a reciprocal selection mechanism (coevolution) between the viral and the cellular populations, resulting in either a completely virus-free Py-resistant FL cell line (cure) or in a continuously Py-shedding line bearing Py genome variants. Structural analysis of these viral populations has been carried out, and some viral variants have been isolated and characterized. On the basis of the results obtained, the possible mechanisms of Py persistence in FL cells will be discussed.

In vitro polyomavirus (Py) can establish different virus-cell interactions depending on the type of infected cells (25). Of special interest has been the ability of Py to grow in mouse cells at different levels of cell differentiation. From the earlier work of Solter (21) to recent studies on teratocarcinoma cell lines, a considerable amount of information has been accumulated (for a review see reference 13) defining the host restrictions for Py growth and some of the viral alterations necessary to overcome these limitations (11).

Friend erythroleukemic (FL) cells (for a review see reference 9) are a pleomorphic cell population apparently blocked at various levels of erythroid differentiation. Treatment with appropriate chemicals, however, induces a further erythroid terminal differentiation and results in characteristic morphological and functional changes (e.g., membrane-specific erythroid proteins, induction of globin mRNA, synthesis of hemoglobin, etc.) in 90 to 95% of the cells (6). FL cells thus provide a model system to study the interaction of exogenous virus with host cell differentiation. This type of interaction has already been investigated in FL cells by using herpes simplex virus (16), Py (3), and vaccinia virus (18).

In a previous work (3), we reported that FL cells are susceptible to Py infection in both the differentiated and undifferentiated states. Response to Py and consequent cell death depend on the cellular physiological state. A low percentage of cells survives infection and gives rise to stable lines or clones which can be isolated and established in cell culture as virus-shedding cell lines. Viral particles appear to be continuously produced and released without any significant alteration in cell growth and differentiation. Electron microscopy observations coupled with infective centers (I.C.) measurements indicate that in stable clones only a low percentage of cells (1 to 2%) allows large-scale viral maturation, exhibiting the accumulation of viral particles in nuclear compact aggregates (23). The presence of mature Py particles does not interfere with the maturation of the endogenous Friend leukemia virus.

In this paper we present studies on the establishment of a

persistent infection in FL-Py cells (for a review see reference 17) and on the behavior of FL-Py cells in long-term cultures. Studies on the alterations of virus-cell interaction by anti-Py serum treatment, and analysis of the state of the viral genome in established clones, led us to a possible interpretation of this persistent infection phenomenon. Analysis of FL cells infected by PytsA, a thermosensitive Py early mutant (7), showed that the viral genome can persist in an integrated form at nonpermissive temperature. Py persistence in infected surviving lines generates viral genome heterogeneity and can result in the selection of viral variants with altered genomic structure. Py produced by some of these FL-Py cell clones has been characterized showing specific alterations.

MATERIALS AND METHODS

Cell cultures. FL 745 cells were generously provided by Charlotte Friend. Mouse 3T3 and 3T6 cells, as well as FL cells, were grown in Dulbecco modified Eagle medium supplemented with 5% fetal bovine serum at 37°C in 10% CO₂. Cell death was determined by trypan blue staining. FL cells were cloned in soft agar (0.4%), and I.C. measurements were carried out by overlaying 1 ml of soft agar containing FL cells on a 3T3 monolayer (5-cm-diameter dishes); after 8 to 12 days of incubation, the numbers of plaques were determined by neutral red staining, by the procedure normally used for viral titrations. The percentage of I.C. was expressed as plaques per plated FL cells. Anti-Py serum was obtained from Mike Fried, Imperial Cancer Research Fund, London and was used at a concentration blocking over 1,000 Py PFU. Reconstruction experiments showed that this concentration prevents any cytopathic effect in 3T3 cells with infection carried out at multiplicity of infection = 1,000 PFU/ml.

Py infection and titration. PyA2 (wild type [wt]) and PytsA strains were propagated on 3T3 or 3T6 cells at low multiplicity. Virus titrations were carried out on 3T3 by standard plaque assays. FL cell infections were routinely performed at multiplicity of infection = 50. Virus production was determined by the following procedure: cells and culture

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medium were collected from an FL culture grown to saturation (about 2×10^6 cells per ml); cells were disrupted by freezing and thawing (three times), and plaque assays were carried out on 3T3 monolayers. Reconstruction experiments showed no interference of Friend leukemia virus with titration of Py on 3T3 cells.

DNA extraction. Viral DNAs were extracted by the Hirt selective procedure (10). Total cellular DNAs were extracted by a slight modification of the procedure described by Blattner et al. (1). Briefly, cells were lysed by 1% Sarkosyl-10 mM EDTA solution; the lysates were incubated 2 h with RNase-A (20 $\mu\text{g/ml}$) at 56°C and then with proteinase K (100 $\mu\text{g/ml}$) at 37°C overnight. After incubation, the samples were dialyzed 24 h against 100 volumes of 10 mM Tris (pH 7.6)–1 mM EDTA–1 M NaCl (at room temperature) and then against several changes of 10 mM Tris–1 ml of EDTA (at 4°C).

Restriction endonuclease digestion and blot hybridization. DNAs from different FL-Py clones were digested with various restriction endonucleases under the conditions specified by the manufacturer (New England Biolabs, Beverly, Mass.). Restriction DNA fragments were separated on 1% or 1.6% agarose gels (run into acetate electrophoresis buffer); DNA was transferred on cellulose filters (BA 85; Schleicher & Schuell Inc.), and filter hybridizations were carried out by the Southern procedure (22). ^{32}P -labeled DNA probes were prepared by standard nick translation procedures (19); both purified Py DNA (from virions) and cloned plasmids containing Py sequences were used as probes in blot hybridization experiments.

Plasmid construction. Whole Py DNA and the *HpaII*-3/5-*Bam*HI fragment (from nucleotides 4,632 to 5,292) were cloned into the pAT-153 vector plasmid (26), in the plasmid *Bam*HI site and the *Bam*HI-*Cla*I large fragment of the vector, respectively. All cloning procedures were the same as described in Maniatis et al. (15).

RESULTS

Response of FL cells to Py infection and establishment of persistent infection. Infection of FL cells by Py results in the production of Py particles and the lysis of the large majority of infected cells; the percentage of surviving cells (2 to 10%) depends on the physiological state of the cell culture at the moment of Py infection (3). The surviving fraction did not significantly change when infection was carried out at a multiplicity of infection ranging between 5 and 500.

The results obtained when FL cells were infected by wt Py (at multiplicity of infection = 50) and anti-Py serum was added or removed at different times postinfection (p.i.) are displayed in Fig. 1. The removal of anti-Py serum at 1, 4, or 8 days p.i. resulted in an immediate cell death (Fig. 1A). When, instead, the anti-Py serum was removed at 19 or 25 days p.i., no detectable cell death was observed, and the resulting cell populations (as well as their parental line) were negative for Py I.C. measurement (carried out 44 days p.i.) and fully susceptible to Py infection. In a symmetrical experiment (Fig. 1B), the Py-infected FL cells were grown in the absence of anti-Py serum, and samples of these cells were removed at 3, 5, and 8 days p.i. and transferred to a medium containing anti-Py serum. As expected, addition of anti-Py serum resulted in the recovery of the Py-infected cells. I.C. measurement and clone analysis performed right after infection allowed us to conclude that the primary infection had involved all of the cells. Taken together, these results indicate that a continuous reinfection in the FL-Py

FIG. 1. Response of FL cells to Py infection. Effect of addition or deprivation of anti-Py serum at various times from infection. The continuous lines represent the FL-infected cells grown always in anti-Py-containing medium (A) or in normal medium (B). Arrows indicate when a sample of the culture was shifted from anti-Py-containing medium to normal medium (A) or vice versa (B). The percentage of surviving cells was measured by trypan blue staining.

culture is essential for the recovery of a persistently infected cell population; otherwise (anti-Py-treated line) the recovered population is identical to parental FL cells.

When FL cells were infected by PytsA and kept at permissive temperature (32°C), the same results were obtained. Shifting of the infected FL-PytsA culture to nonpermissive temperature (39°C), carried out 3 days p.i., prevented cell death, but a further shift to 32°C after 18 days of incubation at 39°C resulted in massive death of the culture (Fig. 2). This can be explained by the fact that PytsA does not replicate at 39°C and, therefore, does not apply any selection pressure on FL cells; consequently, after the shift to 32°C the remaining viral copies can begin a new lytic cycle on an FL cell population fully susceptible to Py growth.

These results support the hypothesis that FL cells are a stochastic population for permissivity to Py growth. The primary infection of a single cell does not invariably result in immediate viral production, as supported by the lack of cell killing if anti-Py serum is added up to 12 h p.i. (data not shown). A first viral burst seems to be necessary to cause massive cell death in the infected FL culture. When the Py-infected FL cells are maintained in culture for more than 3 weeks p.i., however, a Py-shedding FL cell line with normal growth rate and no significant cell death becomes established.

Long-term analysis of Py-carrying clones. Six days after Py infection 20 FL-Py clones were isolated in agar. Cultures derived from these isolated clones were tested for viral production ca. 25 days p.i. The results (Table 1) show that all of the FL-Py clones examined produce considerable amounts of Py particles, although there was a noticeable variation between the individual clones with respect to viral titers. We selected six FL-Py clones which were further cultured for several months and monitored for Py production, I.C. measurement, and response to Py superinfection. The results (Table 2) indicate that Py can persist in these FL-Py clones for long periods with high viral titers. The continuous culturing of FL-Py causes a slow but constant decrease of Py production, which is likely due to a decrease in the number of FL cells able to allow Py maturation. Spontaneously cured FL-Py clones, defined as cell lines which were no longer Py producers, could be obtained only after a long

FIG. 2. Response of FL cells to PytsA infection. FL cells infected at 32°C were shifted (after 3 days) to nonpermissive temperature (39°C) and grown for a period of 18 days; at this time a further shift to permissive temperature in the absence (●) or in the presence (▲) of anti-Py-containing medium was performed. Open circles (○) represent the death of PytsA-infected FL cells not shifted to 39°C.

cultivation. I.C. analysis indicated that within the Py-shedding FL clones only a small number of cells (2 to 10%) produced infective particles at any given time.

The persistence of Py infective particles in FL-Py clones without any significant cell death or growth alterations led us to analyze their response to Py superinfection, after 2 months of continuous culture, by monitoring cell death and viral production. The results (Table 2) showed that the FL-Py clones were virtually resistant to Py superinfection and that the small percentage of producer cells could be only slightly increased.

As reported in Table 2 spontaneously cured FL-Py (FL-Py 9) clones could be isolated after a long period of cultivation; in these clones no Py DNA could be detected by Southern blot analysis (data not shown). Cell cure could also be obtained after addition of anti-Py serum to the culture medium. Two FL-Py lines decreased Py production rapidly when the cells were grown in the presence of anti-Py serum (Fig. 3). These data indicate that continuous reinfection plays an important role in Py production by FL-Py cells. Nevertheless, the anti-Py-induced cure needed a longer period of time than could be predicted if reinfection of a small fraction of permissive FL cells is the only way of Py propagation in FL-Py cultures. The complete cure was, in fact, reached 45 to 60 days after anti-Py treatment application (i.e., after 70 to 95 cell duplications in anti-Py containing medium). At this time, no Py DNA could be detected by Southern blot analysis (data not shown).

To determine the biological properties of the spontaneously cured and of the anti-Py cured clones, we tested their response to Py superinfection. The results (Table 3) indicate that Py infection of a spontaneously cured FL clone (FL-Py 9) did not produce detectable cell death, although small amounts of viral particles were produced in the early stages of the infection. Anti-Py-induced cure had two different effects. When antiserum was added just after infection, it originated a culture fully as sensitive as the parental uninfected line. Conversely, when anti-Py treatment was carried out on a stabilized FL-Py clone (FL-Py 78), the resulting cured cells were only partially killed (20%) by Py infection, and the amount of Py infective particles produced was considerably lower than in the previous case. These data led us to define the Py resistance of the cured FL clones as the absence of extensive cell death after Py infection. It should be stressed, however, that despite the absence of massive cell lysis, infection of cured FL clones results in the production (although limited) of Py particles.

These findings indicate that (i) Py persistence in FL-Py

cells does not depend only on lysis of a limited fraction of cells and continuous reinfection of the cell population, (ii) cured FL cells can be isolated from FL-Py-producing clones after either a long period of cultivation or a delayed treatment with anti-Py serum, and (iii) the spontaneously cured FL cells appear to be resistant to a subsequent Py infection.

State of Py DNA in persistently infected FL-Py cells. The long persistence of Py particles in FL-Py cells grown in medium containing anti-Py serum and the rescue of viral particles in FL infection by the PytsA mutant cannot be explained without postulating a limited cell lysis and continuous reinfection mechanism for Py propagation. To investigate the occurrence of Py genome integration into the host DNA, we analyzed the FL-Py cell DNA by Southern blot technique (22). The results indicated that the high number of free Py DNA molecules obscures the Py genomes that have possibly integrated. To overcome this problem, blot analysis was performed on FL-PytsA cell DNA grown at 39°C to eliminate free Py DNA copies. In cellular DNA no viral sequences could be detected, despite the very large amount of Py DNA observed after the cell culture was shifted down to 32°C (Fig. 4, lanes 1 through 7). The lack of detection of Py genome integrated in FL-PytsA cell DNA at 39°C was likely due to the Py integration heterogeneity within the infected cell population. In fact, when the total DNA from a single isolated clone (FL-tsA8, isolated in agar at 39°C) was analyzed by blot hybridization, the digestion with the restriction enzyme *Bam*HI revealed cellular DNA fragments containing putative integrated Py genome(s) (Fig. 4, lane A). Lane B of the same figure contains, as a control, an equivalent amount of DNA from a Py-transformed rat clone (rat 82) that contains a single integrated Py genome (14). Shifting of FL-tsA8 clone to 32°C and subsequent analysis of cellular DNA by Southern blot analysis also revealed high amounts of free Py DNA copies.

These results suggest that Py DNA can persist in FL cells by a continuous integration excision mechanism and that, in the absence of an active large-T protein, the integrated form becomes stabilized.

Rearrangements of Py DNA in FL-Py cell clones. To determine whether the Py DNA present in FL-Py clones was structurally similar to the parental PyA2 DNA, restriction enzyme analysis of the Py DNA present in the Hirt supernatant (10) from various FL-Py clones was carried out. The Hirt supernatant DNAs were digested with the enzyme *Msp*I and analyzed by blot hybridization with the Py genome as a

TABLE 1. Viral production in two independently isolated sets of FL-Py clones^a

Set 1		Set 2	
Clone	PFU/ml	Clone	PFU/ml
FL-Py 4	3×10^9	FL-Py 72	6×10^9
FL-Py 5	1.5×10^8	FL-Py 73	7×10^9
FL-Py 8	4×10^9	FL-Py 75	1.6×10^{10}
FL-Py 9	10^7	FL-Py 78	5×10^{10}
FL-Py 10	2×10^9	FL-Py 80	3×10^9
FL-Py 13	3.5×10^8	FL-Py 83	1.5×10^{10}
FL-Py 15	2.5×10^8	FL-Py 87	10^{11}
FL-Py 16	10^8	FL-Py 88	10^{10}
FL-Py 18	5×10^{10}	FL-Py 90	1.2×10^{10}
FL-Py 19	3.5×10^9	FL-Py 93	5×10^8

^a All clones show a normal growth rate and are inducible by dimethyl sulfoxide treatment. PFU were measured as Py accumulation in culture media during cell growth to saturation. The plaque morphology was identical to that of parental PyA2 virus.

TABLE 2. Py production decrease and response to Py superinfection during long-term culture in six FL-Py clones

Clone	Time (mo):										
	1		2				3		5		12
	PFU/ml	%I.C.	Before superinfection		After superinfection		PFU/ml	%I.C.	PFU/ml	%I.C.	PFU/ml
			PFU/ml	%I.C.	PFU/ml	%I.C.					
FL-Py 9	10 ⁷	4	10 ⁵	0.5–1	10 ⁸	3–4	2 × 10 ³	<0.2	0	0	0
FL-Py 13	3.5 × 10 ⁸	8	2 × 10 ⁷	1–2	10 ⁸	5–6	2 × 10 ⁵	1–2	10 ⁴	0.5–1	0
FL-Py 18	2 × 10 ¹⁰	11	1.5 × 10 ⁸	4–5	10 ⁹	11	4 × 10 ⁶	5	8 × 10 ⁵	2	5 × 10 ³
FL-Py 78	5 × 10 ¹⁰	14	4 × 10 ⁸	5	ND ^a	ND	3 × 10 ⁶	6	5 × 10 ⁵	1–2	2 × 10 ⁴
FL-Py 87	10 ¹¹	19	2 × 10 ⁹	9	ND	ND	7 × 10 ⁸	11	10 ⁷	3	8 × 10 ⁵
FL-Py 90	1.2 × 10 ¹⁰	9	8 × 10 ⁷	4	ND	ND	5 × 10 ⁶	4	10 ⁵	≈1	ND

^a ND, Not determined.

probe. DNAs of various FL-Py clones showed, in addition to the normal *MspI* pattern, some extra *MspI* fragments (Fig. 5A and B). To determine the nature and the location of these extra fragments, the *MspI* digests of FL-Py Hirt supernatant DNAs were analyzed by further blot hybridization, now using as a probe a recombinant plasmid containing the Py fragment spanning from nucleotides 4,631 to 5,292 (from the *BamHI* site to the *HpaII*-3/5 junction). The results (Fig. 5C) indicated that many of the extra *MspI* fragments hybridized with the Py *BamHI*-*HpaII*-3/5 fragment. These data revealed that some of the genetic changes were located between the origin of viral DNA replication and the region encoding the NH₂ termini of viral structural proteins.

Figure 6 illustrates the restriction analysis of Hirt extracts from FL-Py clones carrying three main interesting viral variants: (i) Py 87, from clone FL-Py 87, that was originally the highest producer clone (Table 1), does not show any alteration in restriction patterns (Fig. 6C); (ii) Py 78, the virus produced by clone FL-Py 78, which presents an insertion within *MspI* fragment 3 and the smaller *BamHI*-*BglII* fragments, and an extra *MspI* site in fragment 1 (Fig. 6A and B); and (iii) Py 90, from clone FL-Py 90, which presents a pattern alteration common to many clones and that seems to be generated early in FL-Py cell culture establishment (Fig. 6C). The typical *MspI* digestion pattern exemplified by Py 90 seems to derive from a rearranged molecule that can be identified as a faster-migrating band in the uncut Hirt extract (Fig. 6D).

To study the fate of the variant molecules, the virus

produced by FL-Py 90 was purified on 3T3 cells from a single plaque. The *MspI* digestion patterns of the Hirt extracts from 10 plaque-purified lysates were shown to be identical to the parental A2 virus, thus suggesting that the variant molecules are not vital by themselves (data not shown).

The finding of many similar Py-rearranged DNA molecules suggests a selective advantage of Py-altered genomes in this persistent infection. In fact, different isolated lines and clones from different experiments have similar rearranged patterns. A detailed analysis of the structural and biological properties of these viral variants will be reported elsewhere (manuscript in preparation).

DISCUSSION

The results reported in this paper indicate that FL cells can establish a persistent infection equilibrium with Py, and long-term cultivation of Py-infected FL cells (FL-Py lines) results in the selection of both Py genome variants and FL cells resistant to Py infection. The first result of FL cell infection by Py is the death of ≈95% of the infected cell population. Massive cell death appears to be due to a secondary infection by the first viral burst (see Fig. 1). After a period of crisis, the surviving FL cells start to grow normally and continue to produce high titers of viral particles for a long period of time (more than 6 months). Analysis of FL-Py lines by in situ hybridization, electron microscopy observations and I.C. assays revealed that ca. 1 to 2% of the cells, at any given time, accumulate high amounts of viral particles (23).

FL-Py clones can become cured by continuous culturing

TABLE 3. Effects of Py superinfection on spontaneous or anti-Py serum-induced, cured cells

Cells ^a	PFU/ml before infection	PFU/ml (% surviving cells) at:	
		5 Days p.i.	14 Days p.i.
FL-9 ^b	<1	10 ⁷ (100)	10 ³ (100)
FL-78-A ^c	<1	10 ⁸ (80)	10 ⁸ (100)
FL-Py line-A ^d	<1	10 ¹⁰ (5)	ND ^e (3)
FL cells ^f		10 ¹⁰ (5)	ND (4)

^a No cells contained Py DNA before infection.^b A spontaneously cured FL-Py clone.^c An anti-Py cured FL-Py clone. The anti-Py treatment was carried out on FL-Py 78 clone 3 months after the initial infection (see Fig. 3).^d An anti-Py cured FL-Py line. The anti-Py treatment was carried out just after Py infection (see Fig. 1).^e ND, Not determined.^f Parental uninfected FL-cell line.

FIG. 3. Decrease of Py infective particles released by anti-Py-treated FL-Py cells. Anti-Py serum treatment was carried out 3 months p.i. in an FL-Py isolated clone, FL-Py 78 (●), and in a mass culture of an FL-Py surviving cell line (■). Decrease of viral production in the anti-Py-untreated FL-Py 78 clone (○) and the FL-Py line (□).

FIG. 4. Blot analysis of Py DNA sequences content into total cellular DNA extracted from PytsA-infected FL cells at various times p.i. Lane 1, DNA extracted 6 days p.i., at 32°C. At this time the culture was shifted to 39°C. Lanes 2 through 5, DNAs extracted 7, 14, 21, and 44 days, respectively, after the shift to 39°C. After 44 days, culture was shifted again to 32°C. Lanes 6 and 7, DNAs extracted 2 and 7 days, respectively, after the shift to 32°C. Lane A, total cellular DNA extracted from clone FL-tsA 8; lane B, (as a control) equivalent amount of DNA from rat 82 cells, a Py-transformed rat clone, bearing a single integrated viral genome. (m) is a Py DNA marker. All the DNAs were digested with *Bam*HI restriction enzyme.

for more than 6 months. The resulting cured FL cells do not appear to contain Py DNA in a free or in an integrated form. The cured FL cells are resistant to subsequent Py infection, since they do not show detectable cell death; nevertheless they are still able to support a limited viral maturation. Cell cure has also been observed when FL-Py-infected cells are grown in the presence of anti-Py serum for more than 45 days. The susceptibility of these anti-Py-cured FL-Py lines to Py growth is maximum (identical to the uninfected parental FL cells) when the anti-Py treatment is applied right after Py infection. An anti-Py cure applied to a stable FL-Py line or clone, however, results in the recovery of a partially Py-resistant FL cell population. This result is consistent with the need for a period of time to allow the selection of resistant variant cells.

We obtained some evidence for Py genome integration in FL cell DNA by using FL cells infected by PytsA mutant. First, experiments of temperature shift showed that Py particles can be rescued from PytsA-infected FL cells grown at 39°C for 44 days. Second, the blot hybridization analysis of a total cellular DNA from a FL-PytsA clone (isolated at 39°C) revealed the presence of viral DNA integrated into the host genome.

Analysis of the structure of the Py genomes in various FL-Py cell lines and clones showed the presence of specific alterations in the viral DNA. In all of the cases analyzed, most if not all of the alterations appear to be located within a region of the viral genome containing the origin of Py DNA replication and the regulatory nontranscribed part of the viral genome. A more detailed analysis of these mutants will be reported elsewhere (manuscript in preparation). Viral mutants in this regulatory region have been isolated in many laboratories by selection for Py growth in undifferentiated

FIG. 5. Blot analysis of Py DNA from Hirt supernatant of various FL-Py established clones. A and B, DNAs from FL-Py clones digested with *Msp*I restriction enzyme and hybridized with whole 32 P-labeled, nick-translated Py DNA. C, the same DNAs as in B, hybridized with a 32 P-labeled, nick-translated plasmid containing a Py fragment (see text) which hybridizes only with the *Msp*I 3 fragment (arrow). Py, the PyA2 wt DNA.

mouse cells, which are not permissive to the wt Py strain (2, 8, 11, 12, 20, 24, 28). All these mutants display rearrangements (from a single base pair addition to extensive insertions and substitutions) that involve the regulatory regions near or within the enhancer region as defined by de Villiers et al. (5) and Tyndall et al. (27). All these mutants are

FIG. 6. Digestion patterns of Hirt extracts from FL-Py clones. Py-DNA variants extracted from FL-Py established clones. *Msp*I (A) and *Bam*HI-*Bgl*I (B) digestion of DNA extracted from clone FL-Py 78 compared with that of PyA2 DNA. Arrows, fragments altered in size. *Msp*I fragment 1, absent in FL-Py 78, is found as two extra bands near *Msp*I fragment 4. (C) Digestion with *Msp*I of viral DNA extracted from FL-Py 87 and FL-Py 90 clones. Arrows, extra bands present in the FL-Py 90 DNA. (D) Uncut Py DNA from PyA2 and Py 90 showing the presence of a faster-migrating molecule (arrow).

characterized by selection for growth in a nonpermissive host, whereas our mutants have been obtained in a cell line permissive for parental wt Py. The selective pressure may be very different in the two cases and may involve different regulatory functions. Furthermore, the finding of recurrent and specific classes of rearranged molecules, involving the origin of replication and the nontranscribed part of the genome, strongly suggests the existence in these cells of a selective pressure that deserves further study.

The selection and persistence of these viral genome mutants support the idea that FL cell and Py interaction must be considered as a dynamic situation of two populations exerting a reciprocal selective pressure. Such dual selection results in the appearance of both rearranged Py genomes and Friend leukemia virus-resistant cell variants. This system provides the opportunity to study events which occur over a wide range of time, offering a unique condition to identify rare modifications of the viral regulatory regions which optimize the interactions with host-specific functions.

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