VIROLOGY 125, 242-245 (1983)

Rat Mutant Cells Showing Temperature Sensitivity for Transformation by Wild-Type Moloney Murine Sarcoma Virus

HIROKAZU INOUE,[1] MASUO YUTSUDO, AND AKIRA HAKURA

Department of Tumor Virology, Research Institute for Microbial Diseases, Osaka University, Yamadaoka, Suita, Osaka 565 Japan

Received June 29, 1982; accepted November 5, 1982

To clarify the cellular target(s) of *onc* gene products of Moloney murine sarcoma virus (Mo-MSV), we isolated seven mutant cells that exhibit temperature sensitivity for transformation by wild-type Mo-MSV from Fischer rat cell line. Five strains of these mutant cells showed normal virus production at the nonpermissive temperature when infected with Mo-MSV, suggesting that viral replication is not affected by these cellular mutations. Four of these mutants were also temperature sensitive (*ts*) for transformation by Kirsten murine sarcoma virus (Ki-MSV), whereas the other three mutants were not *ts*, suggesting that our mutants isolated with Mo-MSV can be divided into two classes as regards temperature sensitivity to transformation by Ki-MSV.

Transforming genes and their products in tumor viruses have been extensively investigated. The transforming (*onc*) genes of RNA tumor viruses are derived from the normal cellular genome by recombination with type C virus (*1-5*). Accumulating evidence indicates that these cell-derived genes are intimately involved in the function(s) required for induction or maintenance of viral transformation. The base sequences of the *onc* gene in various RNA tumor viruses have been clarified, and their products have also been characterized (*6*). However, little is known about the cellular target(s) of these *onc* gene products. Clarification of the cellular targets is most important for understanding the mechanism of transformation by tumor virus.

There are several approaches to determination of the targets. One is to isolate cellular mutants that show temperature-dependent control of expression of viral transformation.

We attempted to isolate mutant cells that exhibit temperature sensitivity for transformation by wild-type Moloney murine sarcoma virus (Mo-MSV) from a Fischer rat cell line. In this paper we report the establishment of seven mutants that are temperature sensitive (*ts*) for focus formation by Mo-MSV and describe some characters of these mutants.

Temperature-sensitive mutants were isolated from clone No. 7 of the rat F2408 line (*7*), which is deficient in hypoxanthine-guanine phosphoribosyl transferase ($HGPRT^-$). For convenience in further characterization of the mutant, the $HGPRT^-$ line was used. Clone No. 7 showed typical properties of the untransformed cell line: the cells had low saturation densities and showed anchorage-dependent growth, as judged by their inability to grow in soft agar.

Confluent cultures of No. 7 cells were mutagenized by ultraviolet light (dose; 175 erg/mm^2). Under the experimental conditions, the survival rate of the cells was approximately 10^{-2}. After irradiation, the cells were trypsinized and then appropriate dilutions of the cells were inoculated into plastic plates (6 cm diameter) with Dulbecco-modified Eagle MEM supplemented with 5% fetal bovine serum. Surviving cells were picked up from well-isolated individual colonies after incubation for 7-10 days at 37°C. The cloned cells were

[1] To whom all reprint requests should be addressed.

0042-6822/83/030242-04$03.00/0

TABLE 1

FOCUS FORMATION OF MUTANT CELLS INFECTED WITH Mo-MSV (Hix)

Cells	No. of foci/plate		FFU/ml		Relative ratio (39°/33°)
	33°	39°	33°	39°	
No. 7	(228, 215, 234)	(99, 102, 96)	1.1×10^6	5.0×10^5	1.00
A754	(86, 81, 83)	(11, 11, 11)	4.2×10^5	5.5×10^4	0.291
B210	(128, 119, 153)	(1, 0, 0)	6.7×10^5	1.7×10^3	0.006
B540	(154, 196, 155)	(3, 0, 3)	8.4×10^5	1.0×10^4	0.026
B543	(95, 85, 83)	(0, 0, 0)	4.4×10^5	$<10^3$	<0.005
B566	(84, 63, 72)	(1, 0, 2)	3.7×10^5	5.0×10^3	0.003
B812	(70, 62, 79)	(2, 0, 1)	3.5×10^5	5.0×10^3	0.032
B814	(41, 40, 38)	(1, 2, 2)	2.0×10^5	8.3×10^3	0.092

Note. Cultures of No. 7 and of mutants containing 2.0×10^5 cells were inoculated into 6-cm plastic dishes with Dulbecco modified Eagle MEM (DMEM) supplemented with 5% fetal bovine serum. After incubation overnight at 37°, the cultures were treated with polybrene (2 μg/ml) for 30 min, and then 0.2 ml of Mo-MSV (Hix) preparation was added to each culture. After adsorption at 37° for 1 hr, the cultures were covered with DMEM medium containing 5% fetal bovine serum, and incubated at 33 and 39°. On Days 5 and 7, the culture medium was changed to DMEM containing 2% fetal bovine serum. Transformed foci were scored after incubation for 10 days.

divided into two portions, one of which was assayed for focus formation by Mo-MSV (Hix) (*8*) at 39° in 24-hole plastic plates. Clones showing definite decrease of transforming ability at 39° were selected and were retested for focus formation by Mo-MSV at 33 and 39°. Among 2199 surviving clones tested, 7 showed temperature sensitivity for transformation by Mo-MSV. These are listed in Table 1. Clone No. 7 showed slight temperature sensitivity for transformation by Mo-MSV (Hix), but the transforming ability of these mutants at 39° was 10^{-1}–10^{-2} of that of the parental strain No. 7. In preliminary experiments, the transformation of all these mutants was reversible when the temperature of the cultures was shifted from 39 to 33° after 3 days postinfection by Mo-MSV (Hix) (to be published). In our experimental conditions, incubation during 3 days at 33° were enough to complete the transforming process. This temperature-sensitive character of all these mutants except A754 was stable even during long-term blind passages at 37°.

In order to examine whether these cellular mutants affect viral replication, virus production of mutant cells infected with Mo-MSV (IIix) was investigated. As expected, in all five mutant cells except strain B210, the virus was replicated to the same level as in the parent No. 7 cells. As shown in Table 2, the viral yields in these five mutants at 39° ranged from 5.8×10^3 to 1.2×10^4 FFU/ml and that of No. 7 was 1.6×10^4 FFU/ml. In our experimental conditions, the virus titer in culture fluid of No. 7 cells infected with Mo-MSV was

TABLE 2

REPLICATION OF Mo-MSV IN MUTANT CELLS

Cells	Virus yield (FFU/ml)		39°/33°
	33°	39°	
No. 7	1.7×10^6	1.6×10^4	9.4×10^{-3}
A754	ND	ND	—
B210	1.1×10^6	6.2×10^2	5.6×10^{-4}
B540	2.5×10^6	8.0×10^3	3.2×10^{-3}
B543	1.2×10^6	1.2×10^4	1.0×10^{-2}
B566	8.2×10^5	9.8×10^3	1.2×10^{-2}
B812	7.9×10^4	7.0×10^3	8.9×10^{-2}
B814	2.1×10^6	5.8×10^3	2.8×10^{-3}

Note. Semiconfluent cultures of 2×10^5 cells per 6-cm plastic dish were infected with Mo-MSV(Hix) at m.o.i. 2 as for Table 1. The cultures were incubated at 33 and 39° in growth medium (DMEM + 5% fetal bovine serum), and the culture medium was changed every day. After 7 days, the culture fluid was collected and assayed on No. 7 cells.

always lower at 39 than at 33°; this might be caused by the instability of murine retroviruses at high temperature (unpublished data). Mo-MSV (Hix) released from No. 7 and from all of the mutant cells was not temperature sensitive for focus formation. These results suggest that none of the mutants tested except B210 had a mutation of the genes required for replication of M-MSV such as the receptor for virus.

To exclude the possibility that this temperature sensitivity for transformation was caused by a temperature effect on the cell growth of the mutant cells, we tested the growth rates of the mutants at 33 and 39°. As shown in Fig. 1, there were no significant differences in the growth rates between high and low temperature, showing that the low efficiency of transformation of the mutant cells at 39° does not reflect a temperature sensitivity for cell replication. These results suggested that most of the mutant lines had a mutation of the genes participating in transformation by Mo-MSV.

It is known that the origins of the *onc* genes of Mo-MSV and Ki-MSV are different: the former was derived from a mouse, and the latter was from a rat (*5, 6*). Both are able to transform the same rodent cells *in vitro*, and to induce tumors *in vivo*. However, little is known about the interrelation of the cellular factors required for transformation by Mo-MSV or Ki-MSV.

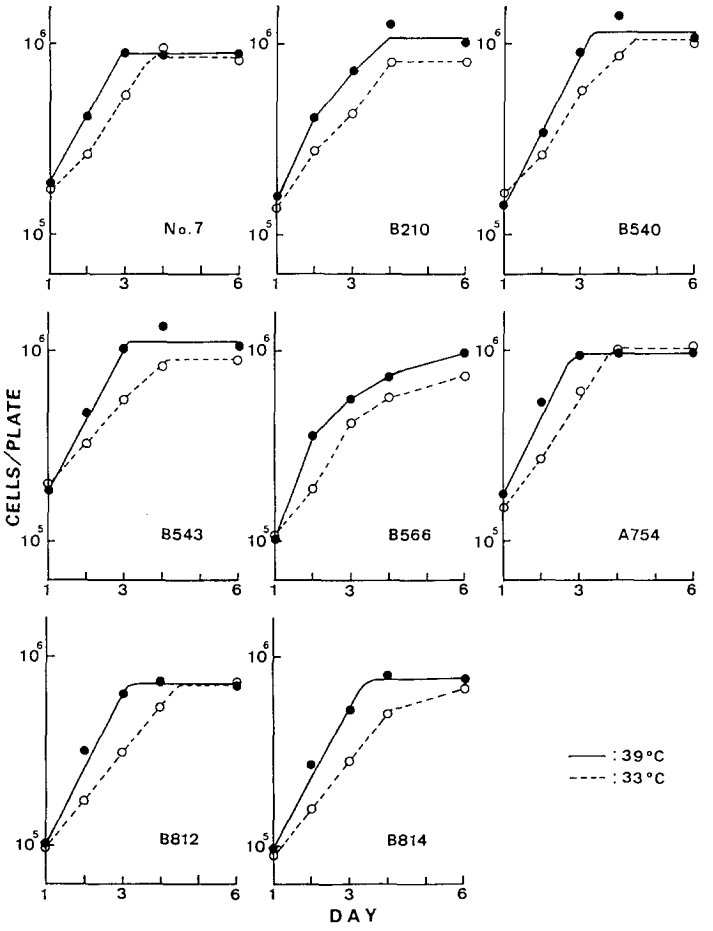

FIG. 1. Growth curves of mutant cells. Exponentially growing cells were inoculated into 3.5-cm plastic dishes (8.0×10^4/plate) with DMEM supplemented with 5% fetal bovine serum and incubated at 33 and 39°. On Days 1, 2, 3, 4, and 6, cell numbers of each cultures were scored. Each value is the average of duplicate cultures.

Thus, it seemed interesting to test whether these mutants also show temperature sensitivity to different *onc* genes from that of Mo-MSV. To examine these relationships, we tested the temperature sensitivity of these mutants to transformation by Ki-MSV. To prepare Ki-MSV having the same viral envelope as the Mo-MSV used in previous experiments, we rescued the virus from KN7-8 (nonproducing cells transformed by Ki-MSV) by superinfection of the Hix strain of MuLV (*8*).

The frequencies of focus formation of the mutants at 33 and 39° were compared after infection with Ki-MSV (Hix). As shown in Table 3, B210 and B566 cells were evidently temperature sensitive for focus formation by Ki-MSV (Hix) as well as by Mo-MSV (Hix), whereas A754, B543, and B814 were not temperature sensitive and were transformed by Ki-MSV (Hix) in the same manner as the parent No. 7 cell at 39°. Two other mutants, B540 and B812, showed weak temperature sensitivity. These results indicated that our cellular *ts* mutants isolated with Mo-MSV (Hix) can be divided into two classes as regards temperature sensitivity to transformation by Ki-MSV (Hix).

Although more information is necessary before making a definite conclusion, most of these temperature-sensitive cells may be mutants affecting the cellular factor(s) required for transformation by Mo-MSV.

TABLE 3

FOCUS FORMATION OF MUTANT CELLS INFECTED BY Ki-MSV(Hix)

Cells	FFU/ml		39°/33°	Relative ratio
	33°	39°		
No. 7	1.0×10^4	1.0×10^4	1.00	1.00
A754	1.1×10^3	2.0×10^3	1.82	1.82
B210	2.5×10^3	1.3×10^2	0.05	0.05
B540	3.7×10^3	5.7×10^2	0.15	0.15
B543	4.5×10^3	3.2×10^3	0.71	0.71
B566	5.8×10^3	3.3×10^2	0.06	0.06
B812	2.4×10^3	4.7×10^2	0.20	0.20
B814	2.4×10^3	2.0×10^3	0.83	0.83

Note. Focus formation was carried out as described in Table 1.

If these cells of A754, B543, and B814 are single, not double mutants, more than one factor must participate in transformation by Mo-MSV. In preliminary experiments, we obtained several hybrids between these mutants and No. 20 (a mutant deficient in thymidine kinase isolated from rat line F2408), which showed the wild-type phenotype with respect to transformation by Mo-MSV.

These mutants would be useful for elucidation of the interrelation of *onc* gene products of Mo-MSV and other tumor viruses, as well as for characterization of cellular targets against Mo-MSV.

Further experiments on the characteristics of the mutants, including complementation analysis between mutants, and temperature-sensitivity tests against other tumor viruses (such as Rous sarcoma virus, Fujinami sarcoma virus, Abelson-MuLV, Polyoma, and SV40) are in progress.

ACKNOWLEDGMENTS

We thank Dr. K. Toyoshima for encouragement and discussions. We also thank Dr. H. Yoshikura for providing the virus and Dr. W. Eckhart for the F2408 cell. This work was supported by the Ministry of Education, Science and Culture, Japan.

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