Ultraviolet Inactivation of Moloney Leukaemia Virus: 
Relative Target Size Required for Virus Replication and 
Rescue of ‘Defective’ Murine Sarcoma Virus

(Accepted 26 October 1971)

Murine leukaemia virus (MuLV) exhibits two kinds of interaction with mouse cells transformed by murine sarcoma virus (MSV) in vitro (Bassin, Tuttle & Fischinger, 1970): an apparent cytocidal interaction accompanied by virus replication and the rescue of infectious MSV. It is not known whether the entire virus genome of MuLV is necessary for these interactions, as has been previously observed in the avian sarcoma-leukaemia system (Levinson & Rubin, 1966). In order to measure the relative target sizes of the genetic information responsible, we studied the kinetics of u.v. inactivation of both the replicating ability of MuLV and the ability of this virus to rescue MSV.

The 3T3FL mouse cell line (Bassin et al. 1970), XC cell line (Svoboda, 1960) and S+L—mouse cell line (Bassin et al. 1970) were employed for virus assays.

For irradiation experiments we employed two Moloney leukaemia virus (MLV) stocks: V9-MV, which contained $7 \times 10^5$ focus inducing units (f.i.u.)/ml. was purchased from Electro-Nucleonics Laboratories, Inc., Bethesda, Maryland, and IC virus (Fischinger, Moore & O'Connor, 1969) that contained $5 \times 10^6$ f.i.u./ml. The source of helper virus in the MSV assay (Fischinger & O'Connor, 1968) was an extract of IC virus-infected mouse spleens obtained from University Laboratories, Inc., Highland Park, New Jersey.

A General Electric germicidal lamp was used for u.v. irradiation. The dose rate was 2200–2400 ergs./cm.²/sec. at a distance of 25 cm. Clarified virus suspensions in phosphate buffered saline (pH 7.2) were irradiated in a watch glass with continuous agitation. Samples were removed at various times and assayed for surviving virus.

Assays for MuLV infectivity were carried out by two separate techniques: focus induction in S+L—cells (S+L—focus assay) and XC plaque assay after infection of normal 3T3 cells. S+L—focus assays were performed in 60 mm. plastic plates (Bassin, Tuttle & Fischinger, 1971a) or in microplates (MicroTest II Tissue Culture Plate, Falcon Plastics, Los Angeles, California). Sensitivity of the S+L—focus assay was compared with the sensitivity of XC plaque assay performed as described by Rowe, Pugh & Hartley (1970). MSV activity was determined by focus formation in 3T3FL cells following the technique of Hartley & Rowe (1966) as modified by O'Connor (1968).

The results of comparative studies of the inactivation kinetics of V9-MV by u.v. irradiation using either the S+L—focus assay or the XC plaque assay are shown in Fig. 1. Virus inactivation followed single-hit kinetics, and no significant differences between the two assay techniques were demonstrated. The 37% survival dose was 22 sec. Non-specific effects of ionization on virus infectivity were not observed in control experiments in which irradiated and non-irradiated medium and phosphate-buffered saline were employed as diluents for titrations of MuLV infectivity.

Infection of S+L—cells with MuLV results in the rescue of infectious MSV. The relative amount of the MuLV genome required to rescue MSV in this system is not known. If only a portion of the genome required for MuLV replication is involved in MSV rescue, MuLV which is unable to multiply following exposure to u.v. light still might be able to rescue
MSV. A microplate technique for focus induction in S+L− cells allowed us to determine the amount of MSV-rescuing activity in MuLV stocks by testing for the presence of MSV in individual wells of S+L− cells infected with dilutions of inactivated MuLV. Microplates previously scored for MuLV focus inducing activity were subjected to two cycles of freezing and thawing to destroy living S+L− cells. Approximately 0.05 ml. of cell lysate from each well was transferred to a new well, and 1 drop of helper virus containing \(2 \times 10^4\) leukaemia virus helper units and 2 drops of 3T3FL cell suspension (1.25 \(\times\) \(10^4\) cells/ml.) were added to each well. After 6 to 8 days' incubation, each well was examined for focus formation. All samples which showed no c.p.e. in the S+L− focus assay produced no MSV foci in 3T3FL cells, and samples which showed c.p.e. in the S+L− focus assay invariably contained MSV transforming activity. Thus, we have been unable to demonstrate the rescue of MSV in the absence of replicating MuLV.

IC virus stocks were then prepared from two different cell cultures in order to test for possible variations in u.v. sensitivity. An IC(3T3) stock was prepared from a cell lysate of chronically infected 3T3FL cells. A second stock, IC(S+L−), was prepared from supernatant fluids of infected S+L− cell cultures. Samples were irradiated in the same manner described above, and surviving MuLV focus inducing activity was assayed. The results, shown in Fig. 2, reveal no differences in inactivation kinetics of the two MuLV stocks prepared from different host cells, and no influence of ‘defective’ MSV which is present in
Fig. 2. Inactivation of Moloney leukaemia and sarcoma virus by u.v. irradiation. Virus suspension of IC(3T3) \((1.8 \times 10^6 \text{ f.i.u./ml.})\) was irradiated at the rate of 2400 ergs/cm.²/sec. Virus suspension of IC(S+L-) \((1.4 \times 10^6 \text{ f.i.u./ml.} \text{ and } 7.0 \times 10^5 \text{ f.f.u./ml.})\) was irradiated at the rate of 2300 ergs/cm.²/sec. Replication of MuLV was determined by S+L- focus assay (Δ in IC(3T3), ○ in IC(S+L-)) and transforming activity of MSV was assayed in 3T3FL cells in the presence of added optimal level of helper virus (●).

S+L- cell lysates on the sensitivity of MuLV to u.v. light. The 37% survival doses of MuLV in IC(3T3) and IC(S+L-) stocks are 29.5 and 30.5 sec., respectively. Fig. 2 also shows that the 37% survival dose of MSV rescued from S+L- cells was 43.5 sec., indicating that the MSV focus forming activity of this stock is about 1.4 times more resistant to u.v. light than is MuLV replicating activity. These same results were obtained in separate experiments with an MSV stock prepared in 3T3 cells.

The ability of MuLV to rescue MSV from S+L- cells and its ability to replicate are equally sensitive to u.v. irradiation. The entire genome of MuLV, therefore, appears to be necessary for the rescue of ‘defective’ MSV. That the induction of MSV from S+L- cells requires replication of MuLV has been further confirmed by Peebles et al. (1971). The results of the u.v. inactivation experiments presented here are consistent with the assumption that MuLV provides the outer coat for MSV as one of the final steps in the formation of infectious virus (Huebner et al. 1966, Aaronson & Rowe, 1970, Bassin et al. 1971). Our data also indicate that MuLV replication is essential for focus induction in S+L- cells by MuLV. It is noteworthy that our experiments provided no evidence that the MSV genome present in S+L- cells can complement or in some other way reactivate a partially inactivated MuLV genome.

Kelloff, Aaronson & Gilden (1970) have shown that the inactivation of the replicating function of the MuLV followed ‘single-hit’ kinetics with an inactivation rate similar to that.
of the transforming function of MSV. Focus formation by MSV was helper-independent under their experimental conditions. In our experiments, the u.v. inactivation rate for MuLV replicating activity was comparable to that reported by these workers, but MSV transforming activity was approximately 1.4 times more radiation resistant. This observed difference in inactivation rate may arise from several possible mechanisms: difference in genome size; difference in assay methods; difference in radiation sensitivity of nucleocapsids; complementation between u.v. inactivated MSV and ‘helper’ MuLV added at the time of adsorption. In our experiments MSV transformation was measured in the presence of a saturating amount of MuLV helper virus; under these conditions foci result from the replication of MSV (Hartley & Rowe, 1966). In the experiments of Kelloff et al. (1970), MSV transforming activity was measured by focus formation in the absence of added MuLV helper virus. Under these conditions, foci result from the replication of transformed cells following non-productive infection with single MSV particles. If there were complementation of genetic material between MSV and MuLV, the target size of MSV transforming activity would appear to be smaller in our assay system. Evidence for duplication of genetic information between the MSV genome present in S+ L− cells and MuLV has indeed been reported (Bassin et al. 1971 b, Fischinger, Schäfer & Seifert, 1971). However, it should be noted that in the rescue experiments, where u.v. inactivated MuLV was plated on both S+ L− cells and normal 3T3 cells, no evidence for such complementation was observed.

Viral Leukemia Lymphoma Branch, and Viral Biology Branch  
National Cancer Institute  
National Institutes of Health  
Bethesda, Maryland 20014  
U.S.A.

REFERENCES


Short communications


(Received 20 September 1971)