A Progress Report
to the
U. S. Department of Energy

STUDIES ON VIRUS-INDUCED CELL FUSION

Period Covered: July 1, 1978 to
July 31, 1979

MASTER

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

Submitted by: Stanley Person, Principal Investigator
Biophysics Program
Department of Microbiology, Cell Biology, Biochemistry and
Biophysics
The Pennsylvania State University
University Park, Pennsylvania 16802

August, 1979

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

ABSTRACT

A small but detectable and reproducible amount of cell fusion occurs for wild type infections of Herpes Simplex Virus type 1 (HSV-1). Mutants of wild type strains can be isolated that cause extensive cell fusion. In both cases the fusion process appears to be characterized by the presence of two opposing activities--the induction of fusion and its subsequent inhibition. For wild type infections, fusion inhibition is the dominant activity and for mutant-infected cells fusion induction predominates. In an effort to understand more about the fusion inhibition process we have determined the extent of fusion in mixed cell populations. Labeled, sparse, infected cells were surrounded by unlabeled, excess, uninfected (or infected) cells to determine if fusion inhibition is active when present in the same cell as fusion factor, when present in cells that do not contain fusion factor, or in both situations. We found that wild type infected cells fuse to the same extent with each other as with uninfected cells. Therefore fusion inhibitor is active when present in the same cell as fusion factor. Other experiments in which the populations were infected with different syn mutants (or with a syn mutant and wild type) indicate that the inhibition activity can also cause a small decrease of fusion when fusion factor and fusion inhibitor are present in different, but neighboring, cells. Marked variations are found in the capacity of different cell types, such as human embryonic lung (HEL) and HEp-2 to support fusion by particular syn mutants. syn20 causes extensive fusion of HEL but little fusion of HEp-2 cells. Labeled, sparse, infected HEL or HEp-2 cells were surrounded with unlabeled, infected HEL or HEp-2 cells to determine if the capacity of cells to support fusion depended on the nature of the infected cell, the uninfected cell, or both. We found that the nature of the infected cell was most important. HEL cells infected with syn20 fused equally well with uninfected HEL or HEp-2 cells, while infected HEp-2 cells fuse poorly with uninfected HEL or HEp-2 cells. The difference in fusion capacity of the two cell types may be due to a difference in a cellular factor affecting the activity of viral, fusion-associated molecules, and not to cell surface differences of the uninfected cells.

PROGRESS REPORT

We are studying cell fusion induced by mutants of HSV-1. Our overall objectives are to identify the viral-specified molecules that cause fusion, and ultimately to understand the nature of the mutations in these molecules that affect the fusion process. Fusion seems to be characterized by both fusion induction and a subsequent inhibition. Some virus mutants also exhibit a host dependence; they fuse Vero or HEL cells, but not HEp-2 cells. For the current granting period we proposed to study the inhibition process and the host-cell dependence. The specific objectives were to determine:

1. if fusion inhibitor is dominant to fusion factor when both are present in the same cell;
2. if fusion inhibitor is dominant to fusion factor when each is present in a different cell;
3. if the difference in fusability of HEL and HEp-2 cells is due to a difference in glycosylation, a difference in cell-surface fusion factor receptors, or to a difference in plasma membrane structure; and
4. the physical location of syn mutations on the HSV-1 genome.

The results of experiments designed to answer these questions are given below.

Results of related experiments form the subject of Appendices I-III. The material in the appendices is:

- Appendix I. Kousoulas, K. G., S. Person and T. C. Holland. Timing of some of the molecular events required for cell fusion induced by Herpes Simplex Virus type 1. *J. Virol.* 27, 505-512 (1978).
- Appendix II. Snipes, W., T. Coohill, S. Person and G. Keller. Pressure controlled CO₂-air system for animal cell culture. *In Vitro* 4, 917-920 (1979).
- Appendix III. Read, G. S., S. Person and P. M. Keller. Genetic studies of cell fusion induced by Herpes Simplex Virus type 1. Submitted for publication.
-

1. Is fusion inhibitor dominant to fusion factor when both are present in the same cell?

For HEL cells infected with a wild type virus (KOS) at a multiplicity of infection (MOI) of 4-10 adsorbed plaque-forming units (PFU)/cell, fusion begins at about 5 h after infection, for incubation at 34°C, but decreases about 2 h later when approximately 20% of the cells have fused. This is one of several different kinds of experiments that is consistent with the expression of a fusion inhibitor after fusion begins. For syn mutants fusion proceeds for a longer time that is dependent on the particular mutant, before fusion ceases. We wanted to determine if fusion inhibitor was active when present in the same cell as fusion factor.

Sparse, wild type infected cells were surrounded with either an excess of uninfected or wild type infected cells. Sparse infected cells were labeled in their DNA with ³H-thymidine prior to infection and identified after infection by autoradiographs. The extent of fusion was indistinguishable when the surrounding cells were infected or uninfected (Fig. 1). The lack of increase of fusion when the surrounding cells were uninfected indicates that fusion inhibitor is dominant when present in the same cell as fusion factor.

2. Is fusion factor dominant to fusion inhibitor when each is present in a different cell?

syn20 infected cells fused less well with wild type infected cells than with uninfected cells (Fig. 2). The fusion kinetics curve for the fusion of syn20 with wild type is biphasic, which is indicative of fusion inhibition. The curve shape for fusion of syn20 with uninfected cells is not biphasic and probably represents a decrease in the probability of fusion between an infected and an uninfected cell at all times throughout infection. If incubation continued the entire monolayer eventually fused. We conclude that fusion inhibitor is active when present in a cell when the fusion factor is in an adjacent cell.

Figure 1. Fusion inhibition when the fusion factor and fusion inhibitor are present in the same cell. HEL cells prelabeled in their DNA with ^3H -thymidine were harvested, seeded in 16.4 mm diameter culture vessels at a low confluency (approximately 1%) for 6 h and infected at an MOI of 10 PFU/cell. After 0.5 h incubation to allow adsorbed viruses to penetrate, cultures were treated with 1% (v/v) immune serum globulins (isg) for 1 h to inactivate extracellular virus. Unlabeled, uninfected or infected (MOI = 10) cells were harvested and added to 70% confluency along with 0.2% isg and the incubation was continued. At various times after infection autoradiographs were prepared and radioactive cells scored for fusion with non-radioactive neighboring cells. The fraction of labeled nuclei existing as mononucleated cells was measured. The first symbol (KOS) refers to labeled, sparse, infected cells; the second symbol denotes unlabeled, 70% confluent infected (KOS) or mock-infected (mi) cells. In other experiments both the labeled and unlabeled cells were infected (or one population remained uninfected), harvested and seeded onto culture vessels at the same time. Equivalent results were obtained (data not shown). Isg was necessary because viruses adsorb to the empty culture vessels and subsequently infected the cells added in excess. It also appears that viruses can be transferred from the surfaces of the labeled cells to the unlabeled cells. This surprising result made the experiments time-consuming because a number of different unsatisfactory virus-inactivating agents were employed before isg was used. Control experiments showed that these concentrations of isg used were sufficient to minimize secondary infections.

An interesting result was obtained when sparse syn20 infected cells were surrounded by an excess of tsB5 infected cells. tsB5 is temperature dependent for fusion and for the production of a viral glycoprotein B_2 . syn20 causes fusion at the permissive and non-permissive temperature and is complemented by tsB5 at the non-permissive temperature. The data are consistent with the hypothesis that tsB5 is mutant in fusion factor activity and syn20 is mutant in fusion inhibitor activity. One expects tsB5 to produce fusion inhibitor with syn20 infected cells to an extent characteristic of wild type infected cells. It did not (Fig. 2). Although fusion was slightly reduced relative to uninfected cells, there was no break in the fusion kinetics curve and most of the cells in the culture ultimately fused. Since the lack of fusion inhibition in this experiment correlates with the lack of production of glycoprotein B_2 , B_2 itself may be the fusion inhibitor. If so, a limiting amount of B_2 causes maximal fusion while both smaller and larger amounts result in less fusion. However, since the production of other glycoproteins was also reduced at the non-permissive temperature, one could argue that it is the decreased production of other glycoproteins that contributes to the decreased inhibition of cell fusion (data not shown).

3. What causes the difference in fusion capacity of HEL and HEp-2 cells?

Some mutants cause the fusion of both HEL and HEp-2 cells while others, such as syn20, cause the fusion of HEL but not HEp-2 cells. To determine the nature of this difference labeled, sparse, syn20 infected HEL or HEp-2 cells were surrounded by an excess of unlabeled and uninfected HEL or HEp-2 cells. Autoradiographs were prepared and labeled cells were scored for fusion with unlabeled cells. We found that the capacity to cause extensive cell fusion depends on the nature of the infected cell and not on the nature of the uninfected cell (Fig. 3). HEL and HEp-2 cells were extensively fused by infected HEL

Figure 2. Fusion inhibition when fusion factor is present in one cell and fusion inhibitor is present in a neighboring cell. Mixed HEL cell populations were prepared and assayed as described in the legend to Fig.1. The first symbols refer to the infecting virus for the labeled, sparse cells (MOI = 10) and the second symbols refer to the unlabeled, excess cells which are mock-infected (mi) or infected with the indicated virus.

Figure 3. The fusion capacity of HEL and HEP-2 cells. An excess of HEL or HEP-2 cells were added to labeled, sparse syn20 infected HEL or HEP-2 cells and assayed for fusion as indicated in the legend to Fig. 1. The extent of fusion was found to depend on the nature of the infected cell and was independent of the nature of the surrounding cells.

cells, but not by infected HEp-2 cells. Since the fusion capacity is independent of the uninfected cell type it is unlikely that the difference in fusion capacity can be explained by a difference in cell-surface receptors for fusion factor for the two cells. Since the difference in fusion depends upon the infected cell, it is more likely due to a difference in a cellular factor that affects viral fusion activity. It could be due either to a cellular enzyme activity (a glycosyl transferase) or to an internal cellular structural protein that alters the fusion-associated activity of a viral glycoprotein. Since syn20 infected HEL cells fused with syn20 infected HEp-2 cells (data not shown) it cannot be argued that the process of infection of HEp-2 cells renders their membranes refractory to fusion.

4. Physical mapping of syn mutants on the HSV-1 genome.

In order to investigate in more detail the capacity of different cells to affect fusion, these experiments were not initiated. They were replaced by studies of the recessive-dominance interactions between syn mutants and wild type viruses.

In preliminary experiments it appeared that the fusion phenotype was dominant in HEL cells, while reports in the literature indicated that the fusion phenotype was recessive in HEp-2 or BHK-21 cells. Therefore, the recessive-dominance interactions of mP and MP were studied in detail for different MOI of the mutant and parent virus for four different cell types. The extent of dominance of MP in mixed infection with mP was determined on monolayer cultures of HEL, CV-1 (Vero), BHK-21, and HEp-2 cells. Labeled and unlabeled cells were mixed in a ratio of 1:70, seeded at 70% confluency, and infected 10 h later at an MOI of 10 or 5 each for mixed infections. At various times after infection autoradiograms were prepared and labeled cells were scored for fusion. (Labeled cells were used solely as random marker cells to be scored for fusion in these

Figure 4. Recessive-dominance interactions of mutant and parent viruses in mixed infections of HEL, CV-1 (Vero), BHK-21 or HEp-2 cells. Fusion kinetics were determined for monolayer cultures infected with a parent virus (mP) and a mutant that causes external cell fusion (MP) at the indicated MOI. MP is dominant to mP in HEL cells but not in the other cell types.

TABLE 1

Cell	Virus	Virus yield (PFU per cell)		Mixed infection Single infection	
		MP	mP	MP	mP
HEL	MP	62	---	2.2	.09
	mP	---	682		
	MP+mP	139	54		
BHK-21	MP	24	---	2.9	.23
	mP	---	150		
	MP+mP	70	35		
CV-1	MP	90	---	2.8	.20
	mP	---	1250		
	MP+mP	250	250		
HEp-2	MP	32	---	2.9	.28
	mP	---	900		
	MP+mP	93	250		

The total input MOI was 10 in single infections and 5 each in mixed infections.

Although there was an increase in the number of MP and a decrease in the number of mP PFU/cell for HEL cells, the same result was obtained for cell-cell types. In addition it was also observed for syn20 infected cells. The relative increase in yield of MP/mP for HEL relative to other cell types is probably not sufficient to explain the differences observed in the kinetics of fusion at the MOI shown.

experiments.) Fusion kinetics curves are shown in Fig. 4 (4 panels). A gradient of the fusion phenotype can be constructed for the four cells at an input MOI of 5 each for mP and MP. The difference in the dominance of mutant over wild type in the four cells may be due to the same factor that determines the cell's capacity to promote or inhibit fusion. It does not seem to be totally explained by the difference in the inhibition of wild type growth (Table 1) or to differences in the synthesis of specific viral proteins or glycoproteins in the mixed infections (data not shown). Both the difference in the capacity of different cells to promote fusion (Fig. 3) and the dominant effect of some mutants (Fig. 4) can be explained by differences in the processing of viral glycoproteins or in internal cell structural proteins that could interact with fusion-associated molecules.

Manuscripts published:

- Kousoulas, K. G., S. Person and T. C. Holland. Timing of some of the molecular events required for cell fusion induced by Herpes Simplex Virus type 1. *J. Virol.* 27, 505-512 (1978).
 Snipes, W., T. Coohill, S. Person and G. Keller. Pressure-controlled CO₂-air system for animal cell culture. *In Vitro* 4, 917-920 (1979).

Manuscripts submitted for publication:

- Read, G. S., S. Person and P. M. Keller. Genetic studies of cell fusion induced by Herpes Simplex Virus type 1.

Manuscripts in preparation:

- Person, S., R. W. Knowles, G. S. Read, T. C. Holland, P. M. Keller and S. C. Warner. Cell fusion and the kinetics of appearance of viral glycoprotein on the cell surface of cells infected with Herpes Simplex Virus type 1.
 Person, S., S. C. Warner, D. Bzik and V. C. Bond. Genetic evidence for the existence of two viral genes that affect cell fusion in HSV-1 infected cells.
 Bzik, D. and S. Person. The capacity of different cells and viruses to alter cell fusion induced by Herpes Simplex Virus, type 1.

Papers presented at meetings:

- Person, S. and S. C. Warner. Genetic evidence for the involvement of two viral genes affecting cell fusion induced by HSV-1.
 August, 1978. Herpesvirus Meetings, Cambridge, England.
 September, 1978. Intern. Congr. for Virology, The Hague, The Netherlands.

Invited seminars:

Cell fusion induced by syncytial-producing mutants of HSV-1. July, 1978.

Workshop on Slow Viruses, National Institutes of Health.

Cell fusion induced by HSV-1: Evidence for the involvement of two viral gene products. August, 1978. Virology Institute, Glasgow, Scotland.

Characterization of mutants of Herpes Simplex Virus that cause cell fusion.

June, 1979. Molecular Biology and Cell Biology, University of Michigan.

June, 1979. Microbiology Program, University of Chicago.

July, 1979. Biology Department, Brookhaven National Laboratory.

APPENDIX

- I Kousoulas, K. G., S. Person and T. C. Holland. Timing of some of the molecular events required for cell fusion induced by Herpes Simplex Virus type 1. J. Virol. 27, 505-512 (1978).
- II Snipes, W., T. Coohill, S. Person and G. Keller. Pressure-controlled CO₂-air system for animal cell culture. In Vitro 4, 917-920 (1979).
- III Read, G. S., S. Person and P. M. Keller. Genetic studies of cell fusion induced by Herpes Simplex Virus type 1. Submitted for publication.

Reprints + Preprints Removed