Volume 304, number 2,3, 221–224 FEBS 11156 June 1992

Acidic pH induces fusion of cells infected with baculovirus to form syncytia

Evgenia Leikina, H. Ongun Onaran and Joshua Zimmerberg

Laboratory of Theoretical and Physical Biology, NICHD, NIH, Bethesda, MD 20892, USA

Received 25 March 1992

The enveloped baculovirus\insect cell system has been used extensively for expression of recombinant proteins, including viral fusion proteins. We tested wild-type baculovirus for endogenous fusion protein activity. Syncytia formation, dye transfer, and capacitance changes were observed after incubating infected *Spodoptera frugiperda* cells in acidic media, consistent with fusion protein activity. Only a short acidic pulse of 10 s is needed to trigger syncytia formation. Identical results were obtained with recombinant baculovirus. This new system is convenient for studying pH activated cell–cell fusion. However, using this enveloped virus to study the mechanism of recombinant fusion proteins requires caution.

Membrane fusion; Viral fusion; Enveloped virus; Cell–cell fusion; Baculovirus expression system

1. INTRODUCTION

Enveloped viruses fuse to host membranes to enter and infect cells. Fusion proteins are viral envelope glycoproteins which mediate membrane fusion. To study the mechanism of protein-mediated membrane fusion, it would be useful to have a simple expression system for a fusion protein which would allow for optical and electrophysiological studies of fusion pore formation, as well as permit genetic alteration of fusion protein sequence. The baculovirus/insect cell system [1] has been used to advantage in electrophysiological studies of recombinant channels [2]. Influenza hemagglutinin [3], VSV G protein [4], and the fusion proteins from measles [5], bovine coronavirus [6], and human immunodeficiency virus [7] have all been successfully expressed in *Spodoptera frugiperda* (Sf9) insect cells using baculovirus, with fusion activity demonstrated as pH-dependent syncytia formation [4–6].

The mechanism of budded baculoviral entry, based upon ultrastructural studies showing fusion, phagocytosis, and endocytosis, and inhibition of infectivity by lipophilic ions, which prevent normal endosomal acidification, is likely fusion of the baculoviral envelope to the endosomal membrane [8]. The 64K envelope glycoprotein of baculovirus has been implicated as a fusion protein [8]. We tested cells expressing baculoviral envelope proteins in the cell membrane for fusion-competence.

Correspondence address: J. Zimmerberg, Bldg. 10, Room 6C101, National Institutes of Health, Bethesda, MD 20892, USA. Fax: (1) (301) 402 0263.

2. MATERIALS AND METHODS

2.1. *Sf9 cell growth and infection*

Sf9 insect cells were grown at 27°C in Grace's medium plus supplements and 10% FBS (TNM-FH medium). For infection, monolayers of Sf9 cells were incubated with either wild-type or β-galactosidase recombinant virus (0.2 ml, 10^6 or 10^7 plaque forming units (pfu)/ml) at 27°C for 1 h. Supernatant fluid was removed, fresh medium was added, and cells were incubated at 27°C for 36 h. Medium and virus were purchased from Invitrogen (San Diego, CA). In the commercial recombinant virus, the β-galactosidase gene was fused to the polyhedrin promoter and the polyhedrin gene was absent (Invitrogen, San Diego, CA).

2.2. *Quantification of syncytia formation*

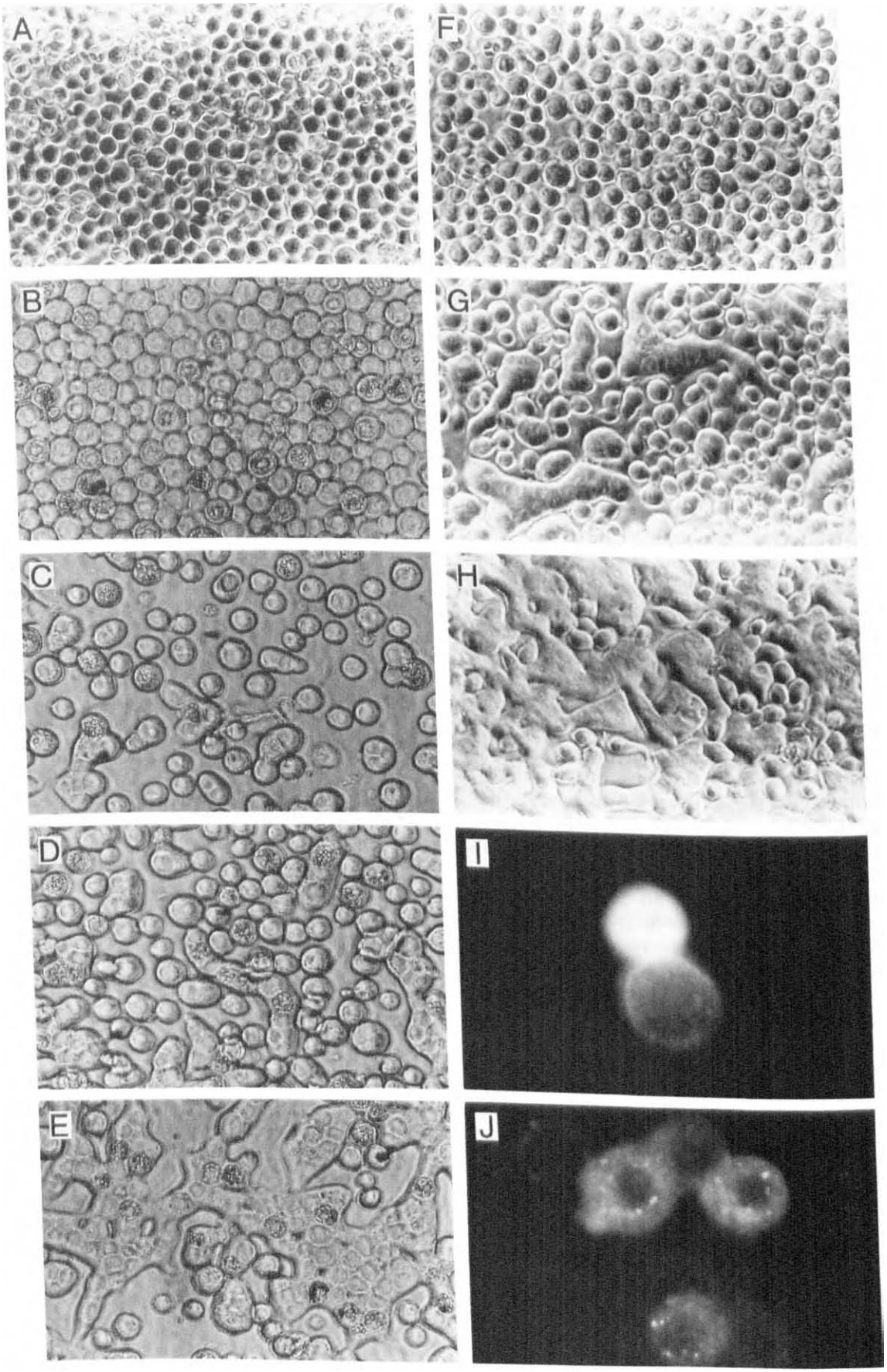
In six high power fields (hpf, 320×), the number of nuclei in syncytia was divided by the sum of nuclei in syncytia and single cells. Capacitance measurements were obtained by reading C_{slow} after capacitance compensation [9].

2.3. *Labeling of cells*

Sf9 cells were labeled by injecting 3.4 mM octadecylrhodamine (R-18, 5 μl per 0.5 ml of medium, Molecular Probes, CA). Cells were washed 5 times with TNM-FH medium, mixed with the same number of unlabeled cells, and placed on a cover glass. Twenty minutes later, the medium was replaced with pH 5.1 medium.

3. RESULTS

Uninfected Sf9 cells did not fuse to each other at any pH. After Sf9 cells (36 h post-infection with wild-type baculovirus) were exposed to acidic media, syncytia formation ensued (Fig. 1A–E). First syncytia formation was observed 2 min after the low pH treatment, and was maximal at one hour. Syncytia formation was graded below pH 5.5, and was maximal at pH 5.2 (Fig. 2A). The efficiency of fusion increased with cell density (Fig. 2A), probably due to increased cell–cell contact. Syncytia formation was also dependent upon viral inoc-


A
F
B
G
C
H
D
I
E
J

ulum and duration of infection. With a high inoculum (10^7 pfu/ml), pH-dependent fusion was seen as early as 20 h after infection. Fusion was also seen as late as 5 days after infection with a low inoculum (10^6 pfu/ml). However, when polyhedra production was maximal, poor syncytia formation was seen (~10%). Recombinant baculovirus did not differ from wild-type baculovirus in any fusion experiment (Figs. 1F–H and 2B). Syncytia formation was preceded by both a commitment step and a membrane fusion step. Exposure of cells to pH 5.1 for only 10 s resulted in significant syncytia formation at one hour (Fig. 2C). Transfer of the lipophilic dyes R-18 and Di-I, as well as rhodamine-labeled phosphatidyl ethanolamine, could be detected well before the morphological changes seen as syncytia in any given pair of cells (Fig. 1I,J). Many pairs of cells could be seen with dye transfer but lacking syncytia. Using cell capacitance to measure surface area, cell–cell fusion was detected by the doubling of surface area for pairs of cells, tripling for three cells fusing together, etc. (Fig. 3A). This quantal capacitance increase which occurred prior to the morphological changes seen as syncytia formation, has the complex admittance characteristic of a true syncytia (Fig. 3B).

4. DISCUSSION

Our results show that cells infected with baculovirus fuse together when exposed to low pH, as measured by lipid mixing, increase of surface area, and formation of multinucleate syncytia. Probably a baculovirus-encoded, pH-dependent fusion protein is expressed in the plasma membrane of infected cells, to be incorporated in the viral envelope after capsid budding for subsequent viral infectivity. Volkman and colleagues have implicated a 64K envelope glycoprotein as fusion protein, and the shift in baculoviral life cycle from budding to polyhedra is accompanied by a loss of a 64K glycoprotein from the viral envelope [8]. Different stage of viral life cycle, cell density, or innoculum may explain the lack of cell–cell fusion seen in other reports [4,6]. Vialard and colleagues report fewer syncytia with wild type than measles-recombinant proteins, testing 72 h after infection [5]. Since they use pH 5.8 to trigger fusion, it is likely that the exogenous fusion protein plays a role. However, using the baculovirus system to study the mechanism or identity of exogenous recombinant

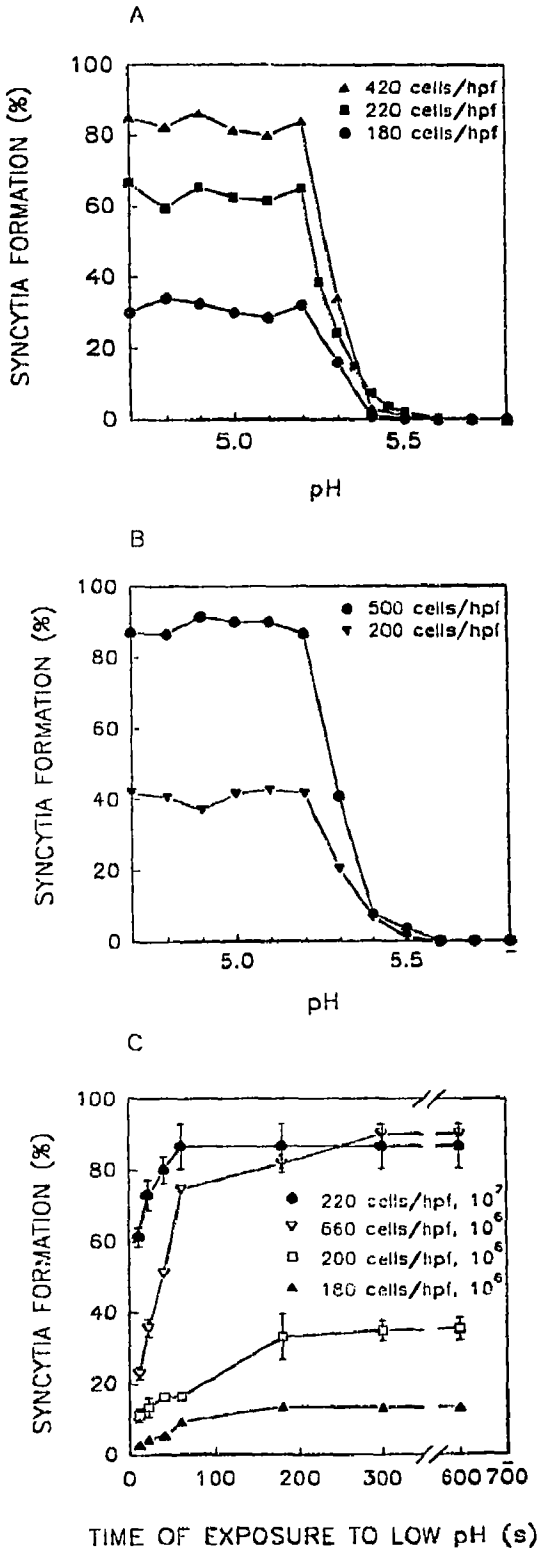


Fig. 2. A,B. To initiate fusion, media bathing monolayers of Sf9 cells infected with either wild-type (A) or β-galactosidase-recombinant (B) baculovirus was replaced with media of differing pH varying from 4.7 to 5.8. The pH of the media were varied by adding 0.1 M citric acid. Syncytia were measured after one hour. C. Monolayers of Sf9 cells infected with wild-type baculovirus (10^6 or 10^7 pfu/ml) were treated with pH 5.1 medium TNM-FH for short times (10 s – 10 min), then returned to normal pH 6.2 medium. After one hour, syncytia formation was measured.

←
Fig. 1. A. Uninfected Sf9 cells in pH 5.1 TNM-FH medium after one hour. (A–E, 660 cells/hpf). B. Sf9 cells infected with wild-type baculovirus at pH 6.4. Polyhedra are evident. C–E. Cells as in B one hour after exposure to pH 5.1 for 10 or 20 s, or one hour, respectively. F. β-galactosidase recombinant infected Sf9 cells in pH 6.4 medium. 500 cells/hpf. G. As in F after one hour at pH 5.1. 200 cells per hpf. H. Cells as in G at higher density, 500 cells/hpf. I,J. R-18 labeled, infected cells 5 min after change of pH to 5.1.

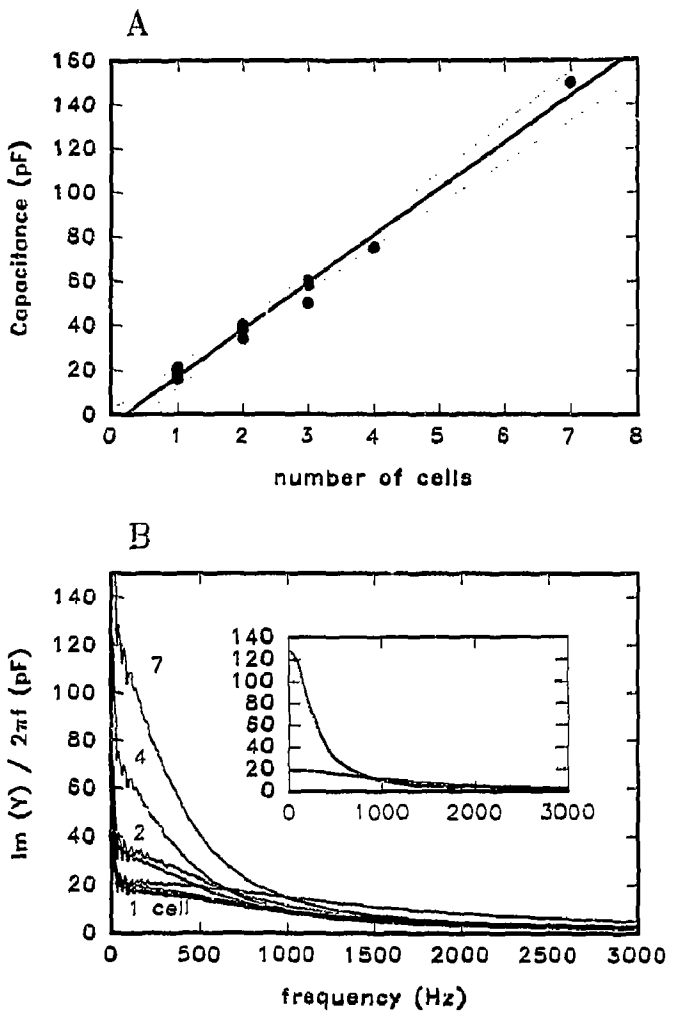


Fig. 3. A. Relationship between whole-cell capacitance, measured 15 min after lowering pH, and number of cells in the cell aggregate which included the patch-clamped cell. Linear regression (shown as a solid line with 95% confidence bands) yields an average value of 21 pF for single cell capacitance, which compares well to that measured for single cells (19 ± 0.9). We used the cell medium as external solution and a KCl buffer as internal solution (150 mM KCl, 7 mM $MgCl_2$, 10 mM HEPES, 1 mM EGTA, pH 7.2). B. The imaginary part of whole-cell admittance, normalized with respect to the angular velocity, is given for a number of cells in mutual contact. The whole-cell recording technique was used on one cell [9]. Zero frequency intercepts estimate different capacitance values before and after lowering pH. Pseudo-random binary voltage signals (0–10 mV), having a quasi-white spectral density (5 kHz bandwidth), were used to determine the complex admittance of the cell membrane [13]. The measured admittance was consistent with the simple equivalent circuit of a series resistance of the pipette and a parallel capacitor/resistor of a single syncytial membrane (inset, showing prediction of this circuit).

viral fusion proteins may be problematic. For example, exogenous fusion proteins may form cooperative units with the endogenous baculoviral fusion protein, or binding may shift pH dependence.

As in influenza HA-mediated cell–cell fusion, constant acidification is not longer needed for subsequent fusion [10]. Cell capacitance changes are seen prior to morphological changes, as in membrane fusion in exocytosis [11]. The fusion demonstrated here may be an extremely convenient system for studying enveloped viral mediated cell–cell fusion.

Acknowledgements: We would like to thank Steven S. Vogel and Leonya Chernomordik for their help with dye labeling and microscopy, and Teresa Jones for her help in using baculovirus.

REFERENCES

[1] Blissard, G.W. and Rohrmann, G.F. (1990) Annu. Rev. Entomol. 35, 127–155.
[2] Klaiber, K., Williams, N., Roberts, T.M., Papizian, D.M, Jan, L.Y. and Miller, C. Neuron 5, 221–226.
[3] Kuroda, K., Hauser, C., Rott, R., Klenk, H.-D. and Doerfler, W. (1986) EMBO J. 5, 1359–1365.
[4] Bailey, M.J., McLeod, D.A., Kang, C.-Y. and Bishop, D.H.L. (1989) Virology 169, 323–331.
[5] Vialard, J., LaLumiere, M., Vernet, T., Briedis, D., Alkhatib, G., Henning, D., Levin, D. and Richardson, C. (1990) J. Virol. 64, 37–50.
[6] Yoo, D., Parker, M.D. and Babiuk, L.A. (1991) Virology 180, 395–399.
[7] Hu, S.L., Kosowski, S.G. and Schaff, K.F. (1987) J. Virol. 61, 3617–3620.
[8] Volkman, L.E. (1986) Micro. and Immuno. 131, 103–117.
[9] Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Eur. J. Physiol. 391, 85–100.
[10] Kaplan, D., Zimmerberg, J., Puri, A., Sarkar, D.P. and Blumenthal, R. Exp. Cell Res. 195, 137–144.
[11] Zimmerberg, J., Curran, M., Cohen, F.S. and Brodwick, M. (1987) Proc. Natl. Acad. Sci. USA 84, 1585–1589.
[12] Summers, M.D. and Smith, G.E. (1987) A manual of methods for baculovirus vectors and insect cell culture procedures, Texas Agricultural Experiment Station Bulletin no. 1555.
[13] Marmarelis, P.Z. and Marmarelis, V.Z. (1978) Analysis of Physiological Systems, White Noise Approach, Plenum Press, New York, pp. 189–193.