A synergistic factor of an insect granulosis virus agglutinates insect cells[1]

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Summary. The synergistic factor (SF), a lipoprotein which enhances in vivo and in vitro baculovirus infections, occurs in the matrix of the occlusion body (capsule) of a granulosis virus of the armyworm, *Pseudaletia unipuncta*. The SF attaches to certain areas on the surfaces of cell plasma membranes of cultured insect cells, resulting in marked cellular agglutination. The minimal amount of SF detectable by agglutination is approximately 3 µg/ml. Cultured cells of 6 out of 10 insect species in 3 orders are agglutinated by the SF, but not those of 2 mammalian species and the erythrocytes of 8 vertebrate species.

The capsule (occlusion body) of the Hawaiian strain (GVH) of a granulosis virus of the armyworm, *Pseudaletia unipuncta*, contains a unique 126-kilodalton lipoprotein[4], the synergistic factor (SF), which enhances per os infections of a nuclear polyhedrosis virus (NPV) in armyworm larvae[5-7]. The mode of action of the SF is in the attachment of enveloped nucleocapsids of the NPV to the microvillus plasma membrane of the larval midgut columnar cell[8-10]. The phospholipid component is essential for the synergistic activity of the SF[4]. Recently, we have shown that the SF also greatly enhances (100-fold) the in vitro infection of the NPV[11]. During this study, we have observed that the SF causes marked cellular agglutination apparently through its attachment to the insect cell membrane. We report herein the results of our study on this unusual capacity of the SF to agglutinate insect cells.

The SF was isolated from GVH capsules and purified by SF antibody-conjugated column chromatography[12]. The SF concentration was determined by the method of Lowry et al.[13]. Cells used in this study are listed in table 1. The source of these cells was as follows: *Mamestra* and *Leucania* cells, J. Mitsuhashi, NIAS, Tsukuba, Japan; *Lymantria* and *Melanoplus* cells, R.H. Goodwin, Montana St. Univ., Bozeman, Montana; *Spodoptera* and *Trichoplusia* cells, M.A. Whitt and J.S. Manning, Univ. California, Davis, California; *Blattella* cells, K.R. Tsang and M.A. Brooks, Univ. Minnesota, Saint Paul, Minnesota; mosquito and mammalian cultured cells, Y. Arcus, Univ. California, Berkeley, California; sheep and rabbit erythrocytes, purchased from Microbiol. Media, San Ramon, California; mouse erythrocytes, freshly prepared; other erythrocytes (glutaraldehyde stabilized), purchased from Sigma Chem. Co., St.-Louis. The agglutinating effect of the SF on cells was based on the agglutination of cells maintained in an insect-cell culture medium. The SF and cells were prepared in Grace's medium, pH 6.5, (Gibco). 0.25 or 0.5 ml of the medium containing the SF was mixed with an equal volume of cell suspension in a test tuber (1 × 10 cm). Control cells were incubated in the medium without SF. Final cell densities in the mixtures were approximately 5–8 × 10^5 cells/ml for the cultured cells and 0.25% (v/v) for the vertebrate erythrocytes. Each mixture was maintained at 26 °C for 3 h, and examined at hourly intervals for cell agglutination.

The effect of SF was first studied in 2 noctuid moth cell lines, NIAS-LeSe-11 SF158 and NIAS-MB-32 SF258 cells. When the SF was added to these cells a noticeable cellular agglutination occurred (fig. 1B). The degree of agglutination was directly proportional to the concentration of the SF. At a high concentration of more than 200 µg/ml, cellular agglutination was observed within 10–15 min. Approximately 3 µg/ml of the SF was needed to cause agglutination with the 2 cell lines. Cellular agglutination by the SF was specifically inhibited with the anti-SF rabbit serum but not with the normal rabbit serum (data not presented). When the SF was heated at 70–80 °C for 10 min, it precipitated, but still retained its agglutinating capacity. Our previous study showed that the SF maintained its enhancing capacity when heated up to 80 °C[14].

Observations with the light microscope showed no cytopathology in cells agglutinated by the SF. There was no polykaryocytosis or cell fusion and growth of the cells was not inhibited. In the electron microscopic study, samples of SF-treated and control cells were centrifuged at 1,000 rpm for 5 min and the cellular pellets were collected. The pellets were fixed, embedded, sectioned routinely, and stained with uranyl acetate and lead citrate. Samples labeled with ferritin were not stained. The SF-treated and control cells showed the following features: (1) the SF-treated cells were tightly attached to each other with little or no visible intercellular spaces (fig. 1D), while the control cells had wide intercellular spaces filled with pseudopodia (fig. 1C), (2) no cell membrane fusion was apparent in the SF-treated cells, and (3) no cytopathological alterations were observed in both the cytoplasms and the nuclei of SF-treated cells.

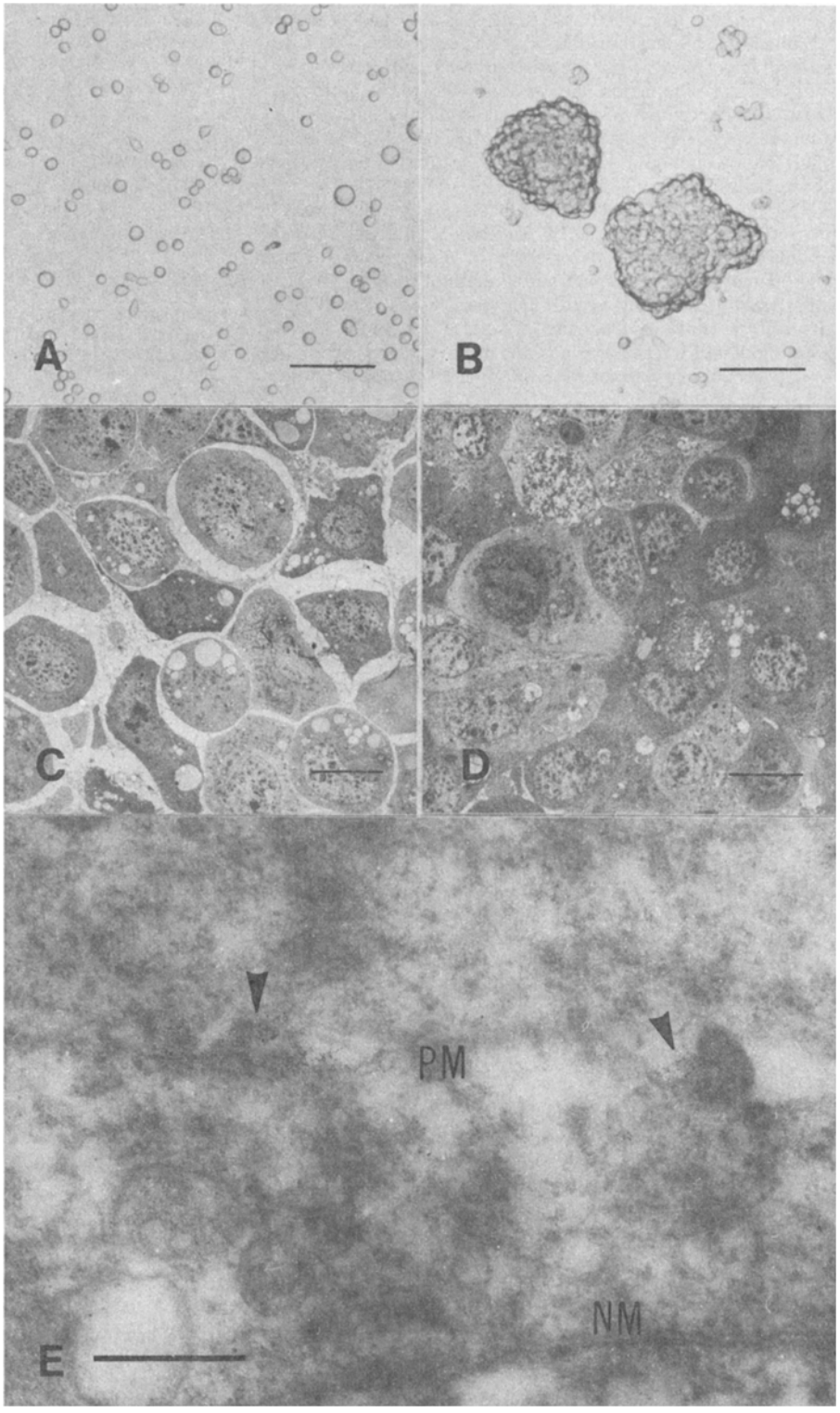
The location of the SF on the treated cells was examined with an indirect ferritin-antibody technique. Cells were treated in turn with the SF, anti-SF rabbit serum, and ferritin-conjugated goat IgG against rabbit IgG (Miles). Control cells were treated in a similar manner except that the SF was not added. Each reaction was conducted at 26 °C for 1 h. Cells were washed 3

Qualitative observations of the agglutinating capacity of the synergistic factor for insect and vertebrate cells

Cell types		Agglutination[a]
Insect cultured cells		
Lepidoptera		
Mamestra brassicae:	NIAS-MB-19 SF278	+ (50)
	NIAS-MB-25 SF225	+ (50)
	NIAS-MB-32 SF258	+ (3-400)
	NIAS-MaBr-85 SF283	+ (50)
Leucania separata:	NIAS-LeSe-11 SF158	+ (3-400)
Spodoptera frugiperda:	SF 21AEII	+ (50)
Trichoplusia ni:	TN368	− (50)
Lymantria dispar:	IPLB-LD-652A	− (50)
Diptera		
Aedes albopictus		+ (50)
Aedes dorsalis		+ (50)
Culex tarsalis		N.D.[b] (50)
Orthoptera		
Melanoplus differentialis:	BRIL-MDF-EP3	− (50)
Blatoidea		
Blatella germanica:	UMN-BGE-2S471	+ (50)
Insect hemocyte		
Pseudaletia unipuncta[c]		− (50)
Mammalian cultured cells[d]		
African green monkey:	VERO	− (50)
Vertebrate erythrocytes		
Sheep, rabit		− (3-200)
Mouse, human (0 group), guinea pig, goat, cow, chicken		− (50)

[a] The degree of cellular agglutination (+ , agglutinated; − , not agglutinated) was examined at the SF concentration (µg/ml) indicated in parantheses. [b] Not determined because of autoagglutination. [c] Cells consisted mostly of spherule cells and autoagglutination hemocytes had been removed prior to use. [d] The cells were treated with trypsin to prepare a homogenous suspension.

times with Grace's medium by centrifugation at 1000 rpm for 5 min for each washing. Cell washings were performed after each treatment. Ferritin-treated cells were processed for electron microscopy. The ferritin particles were not distributed uniformly over the plasma membranes of SF-treated cells, but were apparently restricted to certain regions (fig. 1E). There was no evident ferritin particles on the plasma membrane of control cells. The table presents the results of the agglutination by the SF on cells from insects and vertebrates. The SF agglutinated 1 cockroach, 2 mosquito and 6 lepidopterous cell lines. In these cell lines, autoagglutination occurred occasionally among the cells in culture but, in the presence of the SF, the agglutination was more rapid and pronounced. Even though the SF agglutinated a broad spectrum of insect cell lines, it did not act on all lines tested and showed some specificity. With *Culex tarsalis* cells, positive or negative determination could not be established



Interaction between *Mamestra brassicae* (NIAS-MB-32 SF 258) cells and the synergistic factor (SF). Photomicrographs of (A) untreated cells, and (B) cells agglutinated by the SF (50 µg/ml). Electron micrographs of (C) untreated cells, (D) cells agglutinated with the SF (50 µg/ml), and (E) SF-treated cells stained with ferritin-antibody. PM, plasma membrane; NM, nuclear membrane. Arrow heads indicate location of ferritin particles. Bars in (A) and (B), (C) and (D), and (E) represent 200 µm, 10 µm, and 400 nm, respectively.

because of rapid autoagglutination of the cells. None of the vertebrate erythrocytes and the trypsin-treated mammalian cultured cells were agglutinated by the SF.

Our results indicate that the SF attaches to the surfaces of cell plasma membranes of insect cultured cells. The SF-attaching sites (cellular receptors) are restricted to certain surface areas of the plasma membranes. The agglutinating capacity of the SF suggests the existence on the SF molecule of polyvalent active sites which are responsible for the binding of the SF to cellular receptors on the plasma membrane. Since the SF greatly enhances NPV infection in vitro, our present findings strongly indicate that the SF acts in vitro as an enhancer in the attachment of cell-membrane-budded nucleocapsids of NPV to cultured insect cells as in the case of NPV enhancement in vivo[8-10]. This phenomenon may be analogous to the antibody-mediated enhancement of animal virus infections, such as the enhancement of the virus-specific antibody at a subneutralizing concentration in the attachment of the virus to Fc-receptor-bearing cells[15].

The NPVs are known to cause the hemagglutination of vertebrate erythrocytes[16-20]. Recently, Anderson et al.[21] found that the enveloped nucleocapsids of a GV of *Plodia interpunctella* agglutinated several species of vertebrate erythrocytes, in particular, rabbit erythrocytes. In our present study, the SF did not agglutinate the erythrocytes of several vertebrate species including that of the rabbit. Thus, the hemagglutinin present in enveloped nucleocapsids of *P. interpunctella* GV appeared not to be related to the SF.

The SF agglutinates not only lepidopterous insect cells but also those of mosquitoes and a cockroach. Since the enveloped arboviruses, i.e. *Alphavirus* group, multiply in the vector mosquito, the SF through its effective attachment may enhance the infection of these viruses in mosquito cells in vitro. Such a study may explain, in part, the variations in the efficiency of mosquitoes as vectors.

The basis for the attachment of the enveloped baculoviruses to the host plasma membrane to initiate fusion has not been clarified. Our previous study in vivo suggested that the phospholipids and ionic charges of the SF and viral envelopes were involved in attachment[22-24]. The SF appeared to enhance the attachment and/or fusion of the enveloped virion to the plasma membrane.

The question of virus receptor sites has not been resolved[25]. The adsorption of the SF to certain regions of the plasma membrane suggests the presence of receptor sites on the cell membrane. The attachment of the SF to the host cell membrane and its capacity to enhance the baculovirus infection provide us with a unique opportunity to further investigate this problem.

1 We thank Dr M. Nagata and Dr T. Yamamoto for preparing the synergistic factor and R.T. Hess and E.M. Omi for technical assistance. We also thank the donors who provided the cell lines. This work was supported by the National Science Foundation under Grant No. PCM-8201247.
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0014-4754/84/070742-03$1.50 + 0.20/0

The effect of X-rays and EMS on the behavior of the transposing element, TE98, in *Drosophila melanogaster*

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17 August 1983

Summary. The sensitivity of TE98 (carrying w^{+R} and rst^+) to X-rays does not differ significantly from the mutability of *curled* and *karmoisin* loci. In addition no spontaneous mutants of TE98 were recovered, indicating its extreme stability. On the effect of EMS no *white* mutants were found supporting the view that the w^+ gene of TE98 is duplicated.

Several classes of transposable genetic elements are known in *Drosophila melanogaster*. Similarly to procaryotic transposons, they play an important role in spontaneous and induced mutational events[2]. The mutagen sensitivity of a transposable DNA sequence inserted adjacent to the *white* locus has been demonstrated[3]. Few data are available on the response to mutagenic agents of other *Drosophila* transposons. We report on the behavior of a large transposing element TE (in a position named TE98 in 3R) under conditions of induced mutagenesis.

In the course of a cytogenetic analysis of the 87A–C region of the 3rd chromosome, we isolated a large number of deficiencies induced by X-rays. In these experiments we used the transposing element *TE98* described by Ising and Block[4] as a genetic marker. This transposing element is cytologically visible in polytene chromosome preparations and it carries a piece of the X chromosome with the *white* (w^{+R}) and *roughest* (rst^+) genes. *TE98* is a derivative of *TE1* from which more than 100 new positions have been identified. The transposing elements