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Virus-like particles with host protein-like antigenic determinants protect an insect parasitoid from encapsulation

I. Feddersen, K. Sander* and O. Schmidt+

*Institut für Biologie III, Schänzlestr. 1, and *Institut für Biologie I, Albertstr. 21a, D-7800 Freiburg (Federal Republic of Germany), 30 December 1985*

Summary. Virus-like particles from several species of parasitoid wasps are known to interfere with encapsulation. We raised vertebrate antibodies against the virus-like particles from the wasp *Venturia canescens* and used them to show that antigenic determinants of the particles display similarity to a protein component of the host. When particles on the egg surface are in turn covered with particle-specific antibodies, their protective function is lost, and antibody-treated eggs suffer encapsulation upon injection into larvae of the host, *Ephestia kuehniella* (Lepidoptera).
Key words. Virus-like particles; parasitoid; defence reaction; antigenic determinants.

Foreign particles, for instance parasitoid eggs, brought into the body cavity of insect larvae are usually encapsulated by hemocytes, which form an inactivating coat. Several wasp species are known successfully to circumvent this defense reaction of the host. In some wasp species virus-like particles have been shown to interfere with the host's defense reaction. The particles were recently characterized as a family called polydnaviridae[10]. They are synthesized in the nuclei of the ovarial calyx gland and, in the case of *Venturia canescens*, are secreted on to the surface of the meiotically arrested eggs as they pass through the gland[12]. It is known that eggs are encapsulated if they are depleted of particles or if the particles are experimentally inactivated before injection into the larvae[2,6,8]. In the parthenogenetic ichneumonid *Venturia*, electron microscopic analysis of calyx cells indicates that the particles produced in the nuclei consist of an electron-dense core surrounded by membrane-like structures. On being passaged through the cytoplasm the particles carry an additional membrane, probably from the nuclear envelope, which is left behind when they leave the cell. Particles found on the surface of the egg still appear to have the membrane-like structure acquired inside the nucleus, and therefore this structure seems to constitute an essential part of the particle.

Material and methods. Proteins from tissues. Tissues of *Ephestia* and *Venturia* were dissected manually and homogenized in ice-cold PBS (138 mM NaCl, 2.68 mM KCl, 7.30 mM Na_2HPO_4, 1.47 mM KH_2PO_4, 0.91 mM $CaCl_2$, 0.49 mM $MgCl_2$, pH 7.3).

Virus preparation. Venturia ovaries from 100–150 wasps were dissected. Oocytes were taken from reservoirs of the lateral oviducts and transfered with a Pasteur pipette into a $CsCl_2$ solution to the final concentration of 402.4 mg $CsCl_2$ per ml PBS. After centrifugation in a SW 65 rotor (40 h, 50,000 rpm, 4 °C) the particle band was removed with a drawn-out Pasteur pipette. The particles band at a density of 1.263 g/cm^3. They were diluted 10-fold with cold PBS and pelleted in a Ti 50 rotor (3 h, 35,000 rpm, 4 °C). The pellet was resuspended in 100 µl PBS, and purified particles were stored in liquid nitrogen.

Immunization of rabbits. Antiserum against particles was obtained from rabbits by injecting 2 ml of particles (about 100 µg protein) from a single preparation together with Freund's adjuvant. After 4 weeks the immunization was repeated with the same amount, and 10 days after the second injection 35 ml of blood were collected for serum preparation; the serum was stored in aliquots at −80 °C.

Western blotting. Proteins were electrophoretically separated on 10% polyacrylamide gels as described[5,13]. The separated proteins were transferred to nitrocellulose filters by electroblotting as described[14] using an electrophoresis buffer containing 0.025 M Tris-HCl, pH 7, 0.192 M NaCl and 20% methanol. After preincubation of the filters in blocking buffer (20 mM Tris-HCl, pH 7.5, 0.5 M NaCl 0.05% Nonidet P-40 and 3% bovine serum albumin (BSA)) at room temperature they were incubated overnight at 4 °C in BSA solution containing the antiserum in a 1:100 dilution. The filters were washed for 45 min in blocking buffer without BSA and then incubated for 2 h at room temperature in

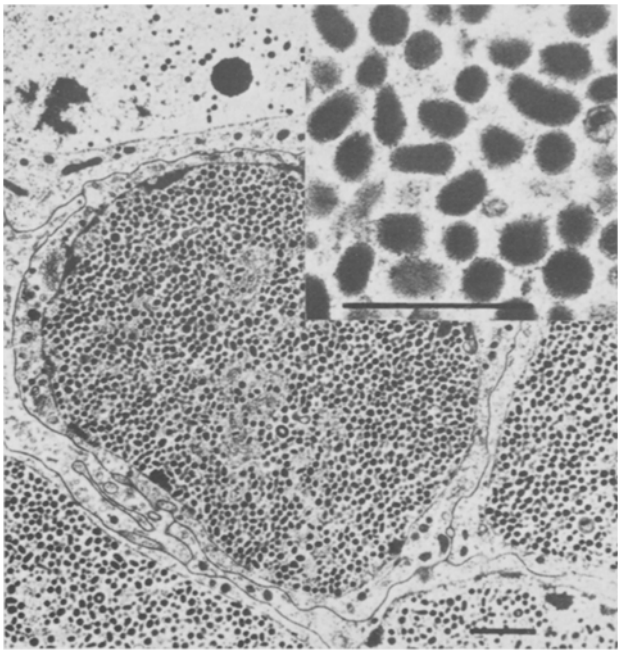

Figure 1. Virus-like particles in the calyx gland of *Venturia canescens*. Electron-dense particles are located predominantly in the nuclei of gland cells. Section shown is from adult wasps. Virus-like particles are already present in calyx cells from pupal stages[12]. Bar represents 1.0 µm (inset 0,5 µm).

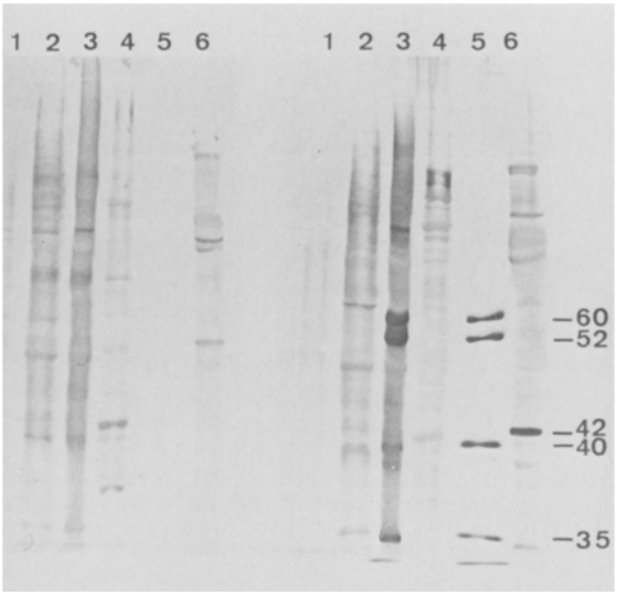

Figure 2. Identification of *Venturia* and *Ephestia* tissue components on Western blots containing 1) *Venturia* accessory glands, 2) *Venturia* brain and thoracic tissues, 3) *Venturia* ovary with calyx gland and oviduct, 4) *Venturia* ovarioles excluding calyx and oviduct, 5) purified virus-like particles, 6) *Ephestia* fat body. Left filter has been screened with rabbit preserum (collected before immunization); right filter with serum from the same rabbit obtained after immunization with purified particles[5]. Molecular weights are given in kDaltons.

anti-rabbit IgG conjugated peroxidase, diluted 1:1000 in blocking buffer without BSA. For visualization of the peroxidase-marked bands the filters were rinsed briefly in a solution containing 5% AEC (3-amino-9-ethylcarbazole, 0.4% dimethylformamide), 95% 0.05 M acetate buffer and 0.1% H_2O_2.

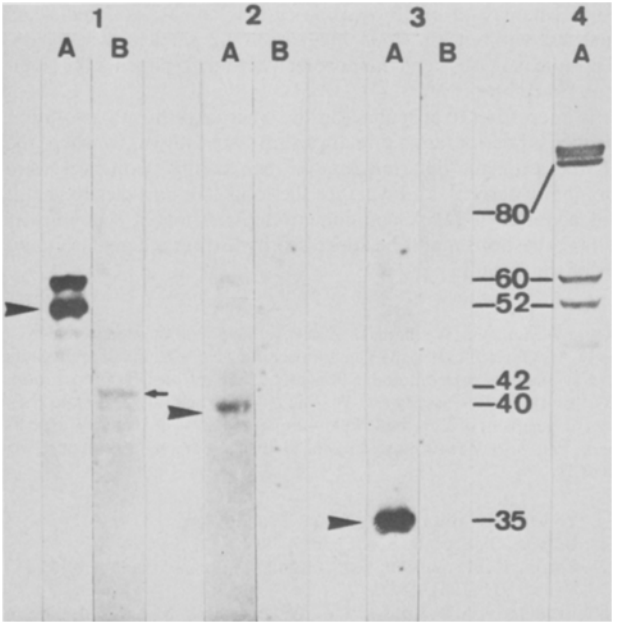

Figure 3. Western blots with ovarial tissues containing virus-like particles (A) and *Ephestia* fat body (B). Each blot containing calyx cells and *Ephestia* fat body was incubated with either purified antibodies eluted from the 52 kD component (1), the 40 kD component (2), the 35 kD component (3) or the 42 kD component of the *Ephestia* fat body (4). The crossreaction to the 42 kD component seen in 1B is weak since incubation is performed in the presence of competing particle antigens from 1A. The molecular weights are given in kDaltons.

Antibody elution. Antibodies were eluted as described by Smith and Fisher[9]. The corresponding bands were excised after peroxidase staining and the small filter fragments were washed briefly in PBS with 0.5% TWEEN 20 (Polyoxyethylene sorbitan monolaurate). The filters were then rinsed three times for 30 s in 5 mM glycine-HCl, pH 2.3, 500 mM NaCl, 0.5% TWEEN 20 and 100 g/ml BSA. The eluates were neutralized with Na_2PO_4 (final concentration: 50 mM). The eluated antibodies were rehybridized to the filters in a volume of about 0.5 ml per cm^2 of nitrocellulose filter.

Results and discussion. Virus-like particles. In order to test for the presence of DNA in the particles, we have carried our several procedures, including attempted DNA and RNA purification from isolated particles, spectroscopic measurements, and determinations of particle density on $CsCl_2$ equilibrium gradients. The results indicate that the particles consist mainly of glycoprotein components with no detectable amount of nucleic acids. This agrees with observations previously reported for *Venturia*[7,10,13], whereas isolated virus-like particles from other wasps contain variable amounts of DNA[2,6,7]. Particles from *Venturia* are found in the nucleus of the calyx gland (fig. 1). Since the electron microscopic structure and the mode of synthesis of both these types of virus-like particles are similar, it seems likely that they are produced in a similar fashion, except that in *Venturia* no DNA is packaged into the particles.

The apparent absence of DNA in *Venturia* particles would preclude any protective function based upon a virus-directed synthesis in the host larva or upon inactivation of its hemocytes by virus infection. However, since the particles coat the egg surface, they could be involved in a passive suppression of the defense reaction if some of their components displayed similarities to host components. In order to test this possibility we purified virus-like particles on a $CsCl_2$ gradient and used these to raise antibodies in rabbits. Antisera and purified antibodies were hybridized to Western blots containing cellular components from various *Venturia* and *Ephestia* tissues.

When purified virus-like particles were separated on acrylamide gels and stained with Coomassie blue, 4 main protein components of 60, 52, 40 and 35 kD mol. wt. were detected, as well as some minor proteins at about 80 kD (not shown). A similar pattern is seen on Western blots stained with particle-specific antibodies (fig. 2). This indicates that all the main protein components carry antigenic determinants. In order to determine the tissue localization of the particle proteins we have tested various tissues from *Venturia* wasps. The results clearly show that only the ovary which included the attached calyx cells contains particle antigens, and other wasp tissues are not recognized by particle-specific antibodies. This indicates that particle-specific antibodies recognize components which are synthesized in substantial amounts only in the calyx gland cells.

When host tissues of unparasitized *Ephestia* larvae were analyzed with particle-specific antibodies, a single protein component with a molecular weight of 42 kD was labeled (fig. 2). In order to rule out coincidental crossreactions in the polyserum we have purified particle-specific antibodies by eluting antibodies from cut-out regions of the Western blot filters containing the peroxidase labeled putative antibodies of the particle components, and incubated them to Western blots[14]. Antibodies which were eluted from the large particle components gave a hybridization signal with the 42 kD host larva protein component (fig. 3). No detectable crossreaction was seen with antibodies eluted from the 40 and 35 kD protein components. The two large components of the virus-like particle must have antigenic determinants in common, since eluted antibodies from each component bind strongly to both. Some weak binding also occurs to the 40 kD component. With antibodies eluted from the small 35 kD component no binding to the three larger components of the virus-like particles is seen.

In an additional experiment we tested purified antibodies from the host 42 kD protein component on Western blots. These

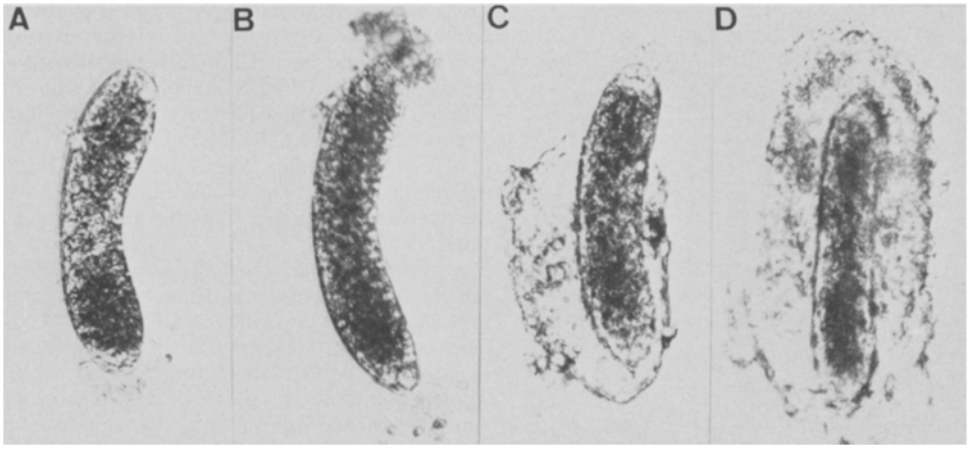

Figure 4. Encapsulation of antibody treated *Venturia* eggs after injection into host larvae. A–D show increasing degrees of cell aggregation around the egg envelopes. Eggs ready for deposition were dissected from the reservoir of the oviduct and incubated for 30 min in a PBS solution containing a 1:10 dilution of the rabbit antiserum against virus-like particles. The eggs were washed several times in PBS and 3-5 each were injected into a host larva with a drawn-out Pasteur pipette. After two days the larvae were dissected in PBS and the eggs recovered.

antibodies bind strongly to both virus particle components of 52 and 60 kD. In addition, a rather strong binding is seen to less abundant protein components of about 80 kD. Apparently, antibodies against these minor components are enriched by the elution procedure. Thus the two major and some minor components of the virus particle share antigenic determinants with a host component which we have found to be localized specifically in the fat body of the host larva. No binding of antibodies was found to proteins of host hemocytes, gut and nerves. Immunofluorescence microscopy shows that the particle antibodies bind to the basal lamella surrounding the host's fat body cells (not shown).

We conclude that the virus-like particles can potentially provide a 'protective coat' to the egg chorion, which then is not recognized as a foreign antigen by the host cells. According to this interpretation of the protection mechanism, if the antigenic determinants of the virus-like particles could in turn be masked experimentally by their respective antibodies, this should provide the egg with a new 'coat' consisting of vertebrate antibodies and consequently cause activation of the host's defense reaction. We tested this possibility in the following way. *Venturia* eggs covered with virus-like particles were dissected from the oviduct, incubated in either serum or preserum of the same rabbit, and then injected into the host larvae. After two days the larvae were opened and the state of encapsulation analyzed. Several degrees of encapsulation were observed as shown in figure 4. Serum and preserum clearly differ in their capability to induce the cellular defense reaction. Eggs completely surrounded by host cells are not observed after treatment with the preserum, whereas a significant proportion of encapsulated eggs is found after serum treatment (table). This indicates a passive function of the virus-like particles in the suppression of the host's defense reaction.

Encapsulation of pretreated eggs after injection into host larvae. Eggs were incubated in each of the following solutions for the indicated times: serum or preserum (30 min), PBS (2 h), 0.1% SDS in PBS (5 min). Before injection with a drawn-out Pasteur pipette into the larvae the eggs were washed several times with PBS. After 2 days the larvae were dissected in PBS and the eggs examined.

	Treatment of eggs			
Host reaction	Serum	Preserum	PBS	0.1% SDS
No visible reaction	12 (13.7%)	52 (82.5%)	51 (100%)	0 –
Aggregation of cells at the posterior or anterior pole	7 (8.1%)	10 (15.9%)	0 –	0 –
Localized encapsulation	7 (8.1%)	1 (1.6%)	0 –	0 –
Complete encapsulation	61 (70.1%)	0 –	0 –	53 (100%)

In an additional experiment we asked whether hemocytes are affected in their ability to encapsulate by a previous injection of particle coated eggs. This was tested by injecting Sephadex beads into the host one day after oviposition by the wasps. The state of encapsulation was tested two days after the injection; all beads were completely encapsulated, whereas the eggs remained unaffected.

We have also tested whether purified particles from a $CsCl_2$ gradient still function as a protective coat. In this experiment eggs from the ovarioles located proximal to the calyx, which are not covered with virus-like particles, were incubated for one hour in a suspension of particles isolated from a $CsCl_2$ gradient (particles from 150 wasps suspended in 300 µl PBS). After injection into the host the eggs were inspected two days later. No signs of encapsulation could be found in any of the 47 recovered eggs, whereas 49 of 52 ovariole eggs were encapsulated when injected without previous incubation in particle suspension. Thus the particles keep their protective function during the isolation procedure.

It is interesting to speculate on the emergence during evolution of such a host/parasitoid relationship, especially as to where the shared antigenic determinants or their coding sequences were originally derived, and whether the virus-like particles have not yet acquired a DNA component or have lost it secondarily. These questions might be addressed by further molecular analysis of the system.

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+ To whom reprint requests should be addressed.

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Further chromosomal studies on *Ellobius lutescens*: Heteromorphism of chromosome No. 1 is not associated with sex determination

M. Djalali, H. Hameister and W. Vogel

Abteilung Klinische Genetik der Universität Ulm, Oberer Eselsberg M25, D-7900 Ulm-Donau (Federal Republic of Germany), 19 February 1986

Summary. Twelve animals of the species *Ellobius lutescens* from two generations were studied with various chromosomal banding techniques. This species carries 17 chromosomes in both sexes. In preceding studies chromosomal sex determination was assigned to different structural variants of chromosome No. 1. In the present study, no definite chromosomal basis for sex determination was found.
Key words. Ellobius lutescens; sex chromosomes; chromosome heteromorphism; sex determination.

The chromosomes of the Persian vole *Ellobius lutescens* Th. (Rodentia Microtinae) were first examined by Matthey[1]. He found an identical karyotype of 2n = 17 chromosomes in somatic and germ cells of both sexes. The smallest chromosome, No. 9, remains unpaired during meiosis in males and females[1]. This chromosome represents 5% of the haploid chromosome complement as does the X-chromosome in most mammals[2]. But chromosome 9 reveals no differences between the two sexes if replication or banding analyses are performed[2,3]. With the aid of banding techniques, two independent samples of *Ellobius lutescens* have been studied[3],[4]. In both of these studies, a correlation between a chromosome No. 1 heteromorphism and sex was observed. However, the heteromorphisms described in these studies were different.

The heteromorphism observed by de la Maza and Sawyer[4] consists of three structurally divergent chromosomes No. 1. The first type of chromosome 1 was found in females as well as in males. The second type was male specific, and the third type was female specific in this sample. The male heteromorphic No. 1 was interpreted as containing material corresponding to a Y-chromosome, and determining male differentiation.

In the study by Wolf et al.[3] identical banding patterns were observed on both chromosomes No. 1 in the male. One of the female chromosomes No. 1 differed from the other by a marked elongation of a proximal band in the long arm. It was proposed that the elongated region of chromosome 1 in females contains a gene which determines femaleness in a dominant way.

At present, our knowledge about naturally occurring chromosome polymorphisms in wild populations of *Ellobius lutescens* is rather limited. Therefore, the interpretations of the results from the small series studied may be premature. The only common feature described in the aforementioned publications is an association between polymorphism of chromosome pair No. 1 and sex. In this study a chromosomal segregation analysis was undertaken on several animals caught in Iran and on their descendants born in this laboratory.

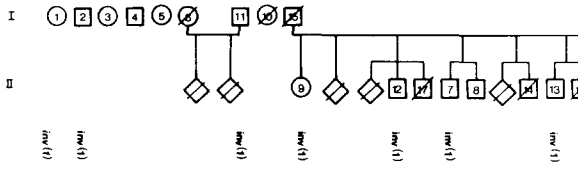

Figure 1. Schematic representation of the *Ellobius lutescens* specimens studied. Laboratory numbers were assigned to the animals according to their date of death. I = animals obtained from Iran, II = offspring gained by breeding. Males and females from our original animal sample (I) sent to us from Iran as well as from breeding (II) were observed to be heterozygous or homozygous for chromosome No. 1 variants. ∅ or ⊠ not karyotyped; inv (1) below the animal symbol means this animal was heterozygous for chromosome No. 1. All other animals were homozygous.

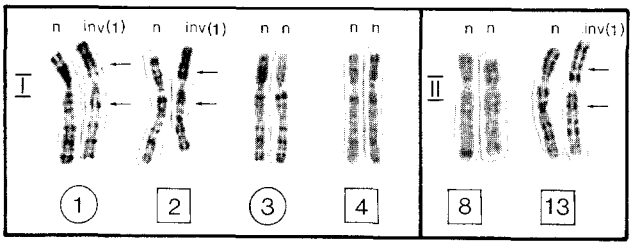

Figure 2. Chromosomes No. 1 of *Ellobius lutescens* with RBG-patterns after fluorescence plus Giemsa staining. Homozygous (n, n) and heterozygous (n, inv (1)) animals were observed in both sexes in generation I (from Iran) and in male offspring (II).

Materials and methods. Six females and four males of *Ellobius lutescens* were obtained from the Pasteur Institute, Teheran, with the help of Dr D. Farhoud, University of Teheran. Five of these animals died within the first two weeks after arrival (animals No. 1 to 5, fig. 1). A sixth animal (No. 10) died later. The remaining two pairs gave rise to several litters of small size (fig. 1), but attempts to rear a third generation were not successful.

Fibroblast cultures were established post mortem according to standard methods[5]. The sex of the animals was verified by histological examination of the gonads. The chromosomes were analyzed from at least twenty mitoses for each animal by Q-bands (QFQ), G-bands (GAG) and replication patterns (RBG). Silver staining of the nucleolus organizer regions (NOR) (Ag-NOR)[6] was also carried out for each individual (fig. 4).

Results. Seven males and five females were investigated during this study (figs. 1 and 2). In all cases the same chromosome number was found (2n = 17). Animals No. 3 (female), 4 (male), 5 (female), 8 (male) and 16 (female) revealed apparently identical pairs of chromosomes No. 1. The banding pattern of this chromosome was designated as the 'normal' type (fig. 2). In the other animals heteromorphism was detected in chromosome pair No. 1. This heteromorphism consists of one normal type chro-