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Fate of polydnavirus DNA of the egg–larval parasitoid *Chelonus inanitus* in the host *Spodoptera littoralis*

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Abstract

In situ hybridizations show that 5 min after parasitization, polydnavirus DNA is in close vicinity of the parasitoid egg, but 5 h later also in the yolk and partially in the host embryo. Fifteen hours after parasitization, the viral DNA is seen all over the host embryo and hardly in the yolk. The tissue distribution of the viral DNA was analysed and quantified by dot blots in the fifth instar parasitized larvae. On a per host basis, haemocytes and fat body contained the highest amount of viral DNA, while nervous tissue, intestinal tract and carcass contained less. Of the three viral segments tested, all were found in all tissues. Relative to the quantity of host DNA, viral DNA was most abundant in haemocytes, about five times less abundant in fat body and nervous tissue and about 25 times less abundant in intestinal tract. The total quantity of viral DNA per host was 444 ± 145 pg which is similar to the quantity injected by the wasp; thus, the viral DNA persists throughout parasitization. The parasitoid larva contains 820 ± 80 pg viral DNA integrated in the genome. This illustrates that the dose of viral DNA injected in virions represents approximately one third of the total viral genomic information present in a host at a late stage of parasitism.

Keywords: Polydnavirus; In situ hybridization

1. Introduction

In many endoparasitoids, polydnaviruses play a crucial role in manipulating the host to the benefit of the developing parasitoid. Two genera are recognized in the polydnavirus family: the bracoviruses associated with braconid wasps, and the ichnoviruses associated with ichneumonid wasps. It is assumed that these two groups evolved from different progenitors (reviewed in Webb, 1998; Webb et al., 2000). Polydnaviruses have a segmented genome of double-stranded circular DNA molecules which replicate from a proviral integrated form in the calyx cells of the wasp's ovary (reviewed in Webb, 1998). Virus particles are injected along with the parasitoid egg into the host and it has been shown that viral DNA persists in the host but does not replicate (Stoltz et al., 1986; Theilmann and Summers, 1986; Strand et al., 1992).

Polydnaviruses play an essential role in protecting the parasitoid from the host's immune system and accordingly, the expression of early viral genes suppressing the immune system of the host has been documented in several hosts of braconid and ichneumonid larval parasitoids (reviewed in Webb, 1998; Schmidt et al., 2001). These are parasitoids which oviposit into larval stages of the host; since the parasitoid egg is immediately exposed to the host's immune system it needs to be protected. In contrast, egg–larval parasitoids oviposit into the egg stage of the host and it is believed that embryos do not yet have a fully developed immune system (Salt, 1968). Thus, in these cases, immediate suppression of the host's immune system is not urgent and the expression of viral genes was found to be low in the early phase of parasitism in the only egg–larval parastioid investigated in this respect (Johner et al., 1999). Polydnaviruses often also interfere with host development (reviewed in Lawrence and Lanzrein, 1993) and stage-specific expression of viral genes has been proposed to be linked with develop-

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mental arrest of the host (Johner et al., 1999; Béliveau et al., 2000; Johner and Lanzrein, 2002).

Only a small amount of information is available on the fate of virions and the distribution of viral DNA within host tissues, and up to now only a small number of larval parasitoids have been investigated. The fate of virions in the host has been studied in a few cases by electron microscopy. For two ichnoviruses it was shown that, approximately 2 h post parasitization, most virions were localized on the outer surface of basement membranes, particularly in the vicinity of muscles. Naked nucleocapsids were seen in cytoplasm and nuclei of mainly fat body, but also muscle, haemocytes and tracheal epithelium. Twenty-four hours after parasitization, no intranuclear virus-related structures could be seen (Stoltz and Vinson, 1979). For two bracoviruses, it was shown that they were associated with basement membranes or in the cytoplasm 45 min to 2 h post parasitization; nucleocapsids were most frequently observed in the vicinity of nuclear pores and were seen most often in fat body, but also in perineurium, midgut, haemocytes, epidermis, Malpighian tubules and muscle cells (Stoltz and Vinson, 1977; De Buron and Beckage, 1992). In one bracovirus, the fate of virions was studied after in vitro infection of haemocytes. In this case, virions were seen attached to the plasma membrane by 15 min and then entered the cells through coated vesicles; empty nucleocapsids were seen in the vicinity of nuclear pores at 2 h post infection (Strand, 1994). The presence of viral DNA in host tissues has been little investigated. According to DNA slot blots, haemocytes appear to be a major tissue containing polydnavirus DNA (Strand et al., 1992). Thus, knowledge of the fate of polydnaviruses and their DNA in the host is still very limited but it appears that viral DNA enters nuclei of host cells rapidly after parasitization and injection of polydnaviruses.

Here, we show the first data on the fate of polydnavirus DNA of an egg–larval parasitoid. As a model system, we are using the bracovirus of *Chelonus inanitus* (CiV) which we have characterized and for which we have shown that individual viral DNA segments are singly encapsidated (Albrecht et al., 1994). We have already sequenced four segments (Wyder et al., 2002) and shown that transcription of viral genes is low in the early stages after parasitization and reaches highest levels towards the end of parasitization (Johner et al., 1999; Johner and Lanzrein, 2002). Here we demonstrate, by in situ hybridization, that viral DNA is first seen in the vicinity of the parasitoid egg and then enters the cells of the host embryo within 5–10 h. By probing dot blots of DNA from various tissues of fifth instar parasitized larvae with total viral DNA or probes made from three different viral DNA segments, we show that haemocytes, fat body, nervous tissue, intestinal tract and carcass all contain viral DNA and that the three tested segments are present in all. Furthermore, we show that the parasitoid larva contains about double the amount of viral DNA in integrated form as is injected in encapsidated form at oviposition.

2. Materials and methods

2.1. Insects

C. inanitus (Braconidae, Hymenoptera) is a solitary egg–larval parasitoid and was reared on one of its natural hosts, *Spodoptera littoralis* (Noctuidae, Lepidoptera). *S. littoralis* was mass reared on artificial diet at 27 ± 1 °C at a photoperiod of 14 h. Adult *S. littoralis* and diet were kindly provided by Syngenta (Stein, Switzerland). For parasitization, 5–8 or 27–32 h old eggs (kept at 20 °C) were used, and after parasitization eggs were placed at 27 ± 1 °C. Under these conditions, the parasitoid first instar larva hatches from its egg 16–22 h after oviposition when the host is still in the embryonic stage. Details of parasitoid and host rearing and their development and morphology are described in Grossniklaus-Bürgin et al. (1994). Designation of embryonic stages was made according to Dorn et al. (1987) who made a detailed analysis of *Manduca sexta* (Sphingidae, Lepidoptera) embryonic development.

2.2. Preparation of eggs and histology

To analyse the fate of the polydnavirus DNA in *S. littoralis* eggs, we used the following procedure. To allow easier handling and orientation approximately ten 5–8 or 27–32 h old eggs were attached with 4% (w/v) calcium alginate (Fluka) in an upright position onto fresh leaves. These eggs were offered to a *C. inanitus* female and oviposition was verified by observation. Eggs were then either analysed immediately (= 5 min post parasitization) or kept at 27 ± 1 °C for 5, 10 or 15 h. Several independent experiments with approximately 10 eggs each were made and nonparasitized eggs were used as controls. Eggs were dechorionated in 2% sodium hypochlorite during 2–3 min and then washed three times in phosphate-buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.4), according to Tautz et al. (1993). The dechorionated eggs were fixed in 4% (w/v) freshly prepared and filtered paraformaldehyde (Merck) in PBS, pH 7–7.5, at 4 °C overnight. After fixation, eggs were washed twice in PBS, dehydrated in an increasing ethanol series, transferred into xylene and embedded in paraffin. The paraffin blocks were cut into 8 or 5 μm sections using a Histo-Range 2218 microtome (LKB). Selected sections were mounted onto poly-L-lysine coated slides (Super Frost Plus, Menzel), dried overnight at 37 °C and stored in a dry atmosphere at −20 °C. Immediately before use, sections were dewaxed two times for 5 min in xylene and

hydrated in a decreasing ethanol series. Some sections were stained with Mayer's haematoxylin–erythrosin (HE).

2.3. Production of probes for in situ hybridization

Probes for in situ hybridization were labelled with Digoxigenin (DIG). Probes were synthesized by PCR using 30 pg plasmid DNA, 200 nM of each primer, 2.5 U of Taq Polymerase (Qiagen) and a PCR DIG labeling mix (Roche) in 50 μl reactions. Thirty-five cycles were performed at 94 and 52 °C for 1 min each and at 68 °C for 1.5 min. For the production of a 242 bp CiV12-specific probe, primers 5′-TCACGATGGCACTTTGCG-3′ and 5′-CAACTCTTGAAATCTGATTCCTTTG-3′ were used with *Hin*dIII-cut clone 1G10. For a 245 bp CiV16.8-specific probe primers 5′-CATGCGCACTGAACGATG-3′ and 5′-AGTCGAG-TAATTGCGAACACCTAAG-3′ were used with *Xho*I-cut clone 2B1. CiV12 and CiV16.8 probes were from non-coding regions (Wyder et al., 2002). To verify complete hydrolysis of mRNA, control experiments were done with a gel-purified 504 bp *S. littoralis* β-actin cDNA fragment (Splactin 5, EMBL accession number Z46873) which was labelled using DIG High Prime (Roche). Unincorporated nucleotides were removed using the QIAquick PCR purification kit (Qiagen).

2.4. In situ hybridization

For in situ hybridization, dewaxed sections were rinsed for 5 min in PBS, covered with 10 mM sodium citrate and exposed to 800 W (15 min) in a microwave oven to permeabilize the cells. The sections were then rinsed with PBS and then freshly prepared 0.07 M NaOH in 70% ethanol was added for 5 min at room temperature (RT) to hydrolyse RNA and denature DNA. Thereafter, 70% cold ethanol (−20 °C) was added to the sample at RT, followed 5 min later by 99% cold ethanol for a further 5 min. For hybridization, 2 μl probe (approximately 50 ng) and 8 μl hybridization buffer (50% deionized formamide, 10% dextran sulphate, 2 × SSC, 50 mM sodium phosphate buffer, pH 7.0) containing 250 ng/μl salmon sperm DNA was placed on the sections. A coverslip was placed on the sections and the edges were sealed with rubber cement. Hybridization was done at 35 °C for 36 h in a moist chamber. Post hybridization washes included three times for 5 min in 50% formamide/2 × SSC (1 × SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0), three times for 5 min in 2 × SSC and once for 5 min in 4 × SSC/0.1% Tween 20 at RT. Sections were then equilibrated for 5 min in DIG-buffer 1 (100 mM maleic acid, 150 mM NaCl, pH 7.5) and blocking was done for 30 min with DIG-buffer 2 (= DIG-buffer 1 containing 1% blocking reagent, Roche). Then, approximately 200 μl of a sheep anti-DIG antibody coupled to alkaline phosphatase diluted 1:500 in DIG-buffer 2 was added for 2 h at RT or overnight at 4 °C. Thereafter, two 15 min washes were made with DIG-buffer 1 followed by equilibration with DIG-buffer 3 (100 mM Tris–HCl, 100 mM NaCl, pH 9.5) for 5 min. Staining was done in the dark at RT in DIG-buffer 3 containing 0.45% (v/v) NBT (nitroblue tetrazolium salt in dimethylformamide) and 0.35% (v/v) BCIP (5-bromo-4-chloro-3-indoyl phosphate, toluidinium salt in dimethylformamide). The extent of staining was regularly checked under the microscope and was stopped after 2–3 d by two washes with TE (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). Sections were embedded in GLYCERGEL™ (Dako). Sections were analysed on a Eclipse E600 microscope (Nikon) using a DXM1200 camera (Nikon).

2.5. Production of probes for DNA dot blots

Probes for DNA dot blots were labelled with [α-^{32}P] dCTP. They were made from total CiV DNA digested with *Hin*dIII and from subclones of segments CiV12, CiV14 and CiV16.8. For CiV12, clone 1G10E1400E330 was digested with *Bam*HI/*Eco*RI; for CiV14, clone 2A6S630 was digested with *Sac*I and for CiV16.8, clone 2B1H0.444B was digested with *Hin*dIII. As a non-viral reference a 504 bp fragment of *S. littoralis* actin was used (Splactin 5, EMBL accession number Z46873). Labelling of gel-purified fragments was done with 50 μCi of [α-^{32}P] dCTP (800 mCi/mmol, Hartmann Analytic) using a random-primed DNA labelling kit (Roche). Unincorporated nucleotides were removed by passage through a Sephadex G-50 column.

2.6. DNA dot blots

For collection of tissues and haemocytes, 8–12 parasitized fifth instar larvae in the feeding stage (9-10-d post parasitization) were used. Larvae were anaesthetized on ice and tissues dissected, with fine forceps, in 0.154 M NaCl. The intestinal tract with Malpighian tubules and the nervous system, consisting of ventral nerve cord and brain, were carefully removed and washed in NaCl. Fat body was collected with broad forceps and washed in NaCl. The carcass with tracheae, muscle, epidermis and some fat body was also collected. Dissected tissues and carcasses were put in ATL lysis buffer (Qiagen) into Eppendorf tubes kept on ice. Whole unparasitized larvae were used as a negative control. Tissues, carcass and whole larvae were homogenized with a Polytron PT 1200 (Kinematica) on level 4 for 45 s in ATL buffer. For collection of haemocytes, 10 larvae were anaesthetized on ice; they were then held in an upright position and the caudal disk was cut away. The outflowing haemolymph was collected in an Eppendorf tube containing some crystals of PTU (1-phenyl-2-thiourea). Haemo-

cytes were pelleted by centrifugation (5 min at 1400 *g*) and suspended in ATL lysis buffer.

DNA was isolated by using the QIamp Tissue Kit (Qiagen) with RNase digestion according to the manufacturer's instructions. Homogenates of intestinal tract, carcass and whole larvae were spun through QIAshredders (Qiagen) after RNase treatment. The concentration of DNA was determined on ethidium bromide-containing agarose plates by comparison with a dilution series of known concentrations of calf thymus DNA. Calyx fluid was collected and the polydnavirus was purified by sucrose gradient centrifugation as described in Soller and Lanzrein (1996). CiV DNA was isolated with the QUIamp Tissue Kit as described above. The concentration of DNA was determined using a photometer (Kontron Uvikon 810) and a dilution series of known concentrations of CiV DNA was made with TE.

Tissue DNA, along with a dilution series of polydnavirus DNA, was denatured, dotted and UV-crosslinked to a positively charged nylon membrane as described (Gruber et al., 1996). Blots were prehybridized for 2–3 h at 42 °C in 5 × SSC, 20 g/l blocking reagent (Roche), 1 g/l lauroylsarcosine, 0.2 g/l SDS, 200 μg/ml denatured calf thymus DNA and 50% formamide. The blots were hybridized at 42 °C overnight in the same buffer which in addition contained 19 ng/ml [α-^{32}P] dCTP-labelled probe. The blots were washed in 2 × SSC, 0.1% SDS at 68 °C and in 0.2 × SSC, 0.1% SDS at 68 °C. The nylon membranes were exposed to a Phosphor Screen (Molecular Dynamics) and quantitatively analysed on a PhosphorImager and the ImageQuant program (Molecular Dynamics). Before reprobing, membranes were stripped by boiling for 10 min in TE containing 0.1% SDS.

3. Results

3.1. In situ hybridizations with parasitized eggs

The fate of polydnavirus DNA in the host egg was studied by in situ hybridization with viral probes at various time points after parasitization. To give an overview, a HE stained section of a host egg, 5 min after parasitization, is shown in Fig. 1a; the developing host embryo, at approximately stage 5 (long, with head appendages and thoracic legs partially developed), and two parasitoid eggs in the yolk are clearly visible. In situ hybridization of a similar section with a viral probe shows two patches of signals in the vicinity of the parasitoid egg 5 min after parasitization (Fig. 1b). No signals were seen when nonparasitized eggs were used as negative controls (Fig. 1c,d). Five hours after parasitization, viral DNA could be seen at various locations (Fig. 2). In Fig. 2a, an HE stained section of a parasitized host egg displays a longitudinal section through the parasitoid egg; it appears that the piercing of the host egg by the parasitoid's ovipositor has left visible traces. A consecutive section analysed by in situ hybridization with a viral probe (Fig. 2b) shows concentrated signals in the vicinity of the parasitoid egg and some scattered signals in the host embryo. Other examples of the situation as seen 5 h after parasitization are shown in Fig. 2c–f. They reveal that viral DNA can be either in the vicinity of the parasitoid egg (c), or in a process of distribution in the yolk (d) or moving towards the host embryo (e) or already distributed in tissues of the host embryo (f). Thus, within 5 h after parasitization, polydnaviruses appear to be in the early process of invading host tissues.

At later stages, namely 10 and 15 h after parasitization, viral DNA was seen mainly in the host embryo and not in yolk. Fig. 3 shows a comparison of HE stained sections (a,c,e) with in situ hybridizations of consecutive sections (b,d,f). At 10 h after parasitization, signals could be seen in large portions of the host embryo (Fig. 3a,b) which had attained stage 7 (embryo shortened). At 15 h after parasitization, signals were seen all over the embryo with either the CiV12 probe (Fig. 3f) or the CiV16.8 probe (Fig. 3d). The host embryo had reached stage 8 i.e. katatrepsis and primary dorsal closure (Fig. 3c,d) or stage 9 i.e. a thin embryonic cuticle is deposited (Fig. 3e,f). More detailed information on the characteristics of the various embryonic stages is given in Dorn et al. (1987).

The signal intensity seen in the in situ hybridizations is not a quantitative measure and great differences were observed even within the same experiment. For this reason, the distribution of the in situ hybridization signal (and not its intensity) is what is important. But, we can exclude the possibility that our experiments detected RNA and not DNA for the following reasons. The viral probes are situated in non-coding regions of CiV12 and CiV16.8 (Wyder et al., 2002), the in situ procedure that we used hydrolyses RNA, and control experiments with actin cDNA as a probe gave only very weak signals (data not shown).

3.2. Analysis of distribution of viral DNA in host tissues by DNA dot blot

The distribution of viral DNA in various host tissues was then analysed 9–10 d after parasitization by DNA dot blots with total CiV or three viral segments as probes (Figs. 4 and 5 and Table 1). Fig. 4 shows an example of a dot blot analysis with CiV, CiV12 and actin as probes and reveals that haemocytes and fat body contain the highest amount of viral DNA, but nervous tissue and intestinal tract also gave clear signals. No signal was seen with viral DNA as probe in a nonparasitized larva (negative control). We do not know why the actin signals are comparatively low in nervous tissue and intestine. A dilution series of CiV DNA (bottom) was used to quan-

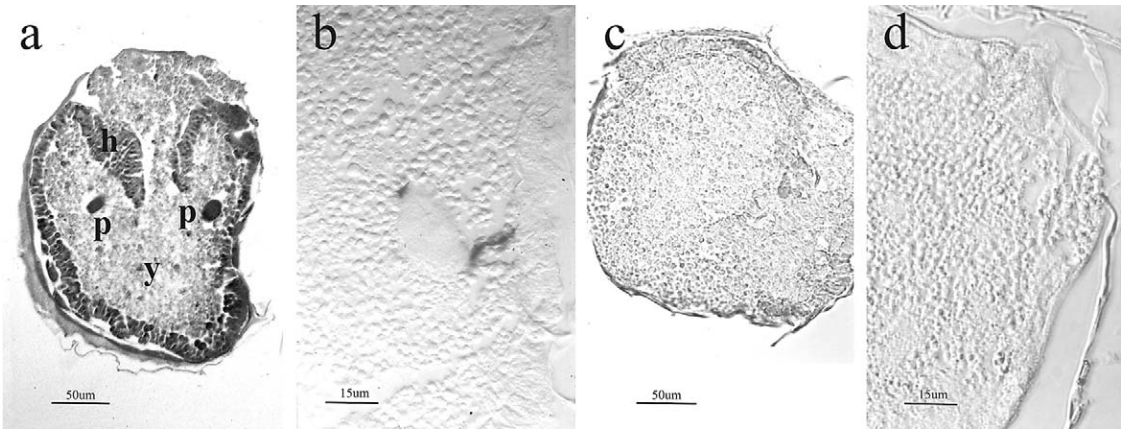

Fig. 1. Sections of 27–32 h old *S. littoralis* eggs. (a) HE stained parasitized egg 5 min after parasitization containing two parasitoid eggs. (b) In situ hybridization with CiV12 probe 5 min after parasitization; the parasitoid egg with two patches of signals next to it are shown. (c) and (d) Negative controls at two magnifications: in situ hybridization with nonparasitized eggs. p, parasitoid egg; h, host embryo; y, yolk.

tify the viral DNA in host tissues. Data in picogram per host and in picogram per microgram host DNA are presented in Table 1. In a fifth instar host larva, the highest quantity of viral DNA is found in fat body and haemocytes and lesser amounts in carcass, nervous system and intestinal tract. The total amount of viral DNA is around 450 pg. With approximately 820 pg, the corresponding parasitoid larva (late L1–early L2) contains about double the amount of viral DNA, in integrated form, of that found in the host. When calculated relative to the quantity of host DNA, haemocytes contain by far the highest concentration of viral DNA, fat body and nervous tissue containing about five times less and intestinal tract having about 25 times less. We also analysed the tissue distribution of CiV12, CiV14 and CiV16.8 and compared it to that of total CiV (Fig. 5). All three segments were found in all tissues analysed and there was no significant accumulation of any one of these segments in one specific tissue. Nevertheless, the distribution of CiV12 and CiV14 was very similar in all tissues investigated while CiV16.8 appeared to be relatively more abundant in intestinal tract and carcass than in fat body and nervous tissue.

4. Discussion

The in situ hybridization data show that polydnavirus DNA of *C. inanitus* is first seen in the vicinity of the parasitoid egg and then moves towards the host embryo (Figs. 1–3). These are the first data on the fate of polydnavirus DNA in the host of an egg–larval parasitoid. As we analysed only viral DNA, we do not know how long this DNA remains encapsidated in virions. From electron microscopic investigations with wasp ovaries we know that virions are not attached to the surface of the parasitoid egg but appear to be injected along with the egg (Wyler and Lanzrein, 2003). This finding is in accordance with the localization of viral DNA in patches near the parasitoid egg by 5 min after parasitization (Fig. 1b); also the fact, that at this time point many sections gave no signals, supports a patchy deposition of polydnaviruses. Five hours after parasitization, viral DNA was seen either mostly in the vicinity of the parasitoid egg, particularly in the egg which was parasitized at an early stage (Fig. 2c), or partly in the yolk near the parasitoid egg and in the host embryo or already mainly within host embryonic tissues (Fig. 2). Ten hours after parasitization, viral DNA was seen only in embryonic tissue and no longer in yolk and 15 h after parasitization the entire embryo appeared to contain viral DNA.

The overall intensity of the in situ hybridization signals was higher by 10 and 15 h after parasitization when viral DNA was in host tissues. This, however, does not mean that the amount of viral DNA increases. Quantities of viral DNA detected per host larva (Table 1) were similar to the quantities injected by the wasp into the egg (Lanzrein et al., 2001). Furthermore, in situ hybridization is not a quantitative method and great differences in signal intensity were observed even within the same experiment. We can exclude the possiblity that our experiments detected RNA as the NaOH treatment used hydrolyses RNA, an effect which was confirmed by control experiments with actin cDNA as probe. In addition, viral probes were situated outside the coding regions (Wyder et al., 2002). Also the great similarity of signals obtained with CiV12 and CiV16.8 as probe (Figs. 2 and 3) supports the postulation that what we see is DNA and not RNA. One possibility to explain the differences in signal intensity in the early phase of parasitization could be a change in accessibility of viral DNA to the probes. It is conceivable that the encapsidated and highly compacted form of viral DNA in virions is less accessible to the probes than viral DNA in host tissues. Under this assumption, one could conclude that viral DNA is encapsidated in virions until host tissues are

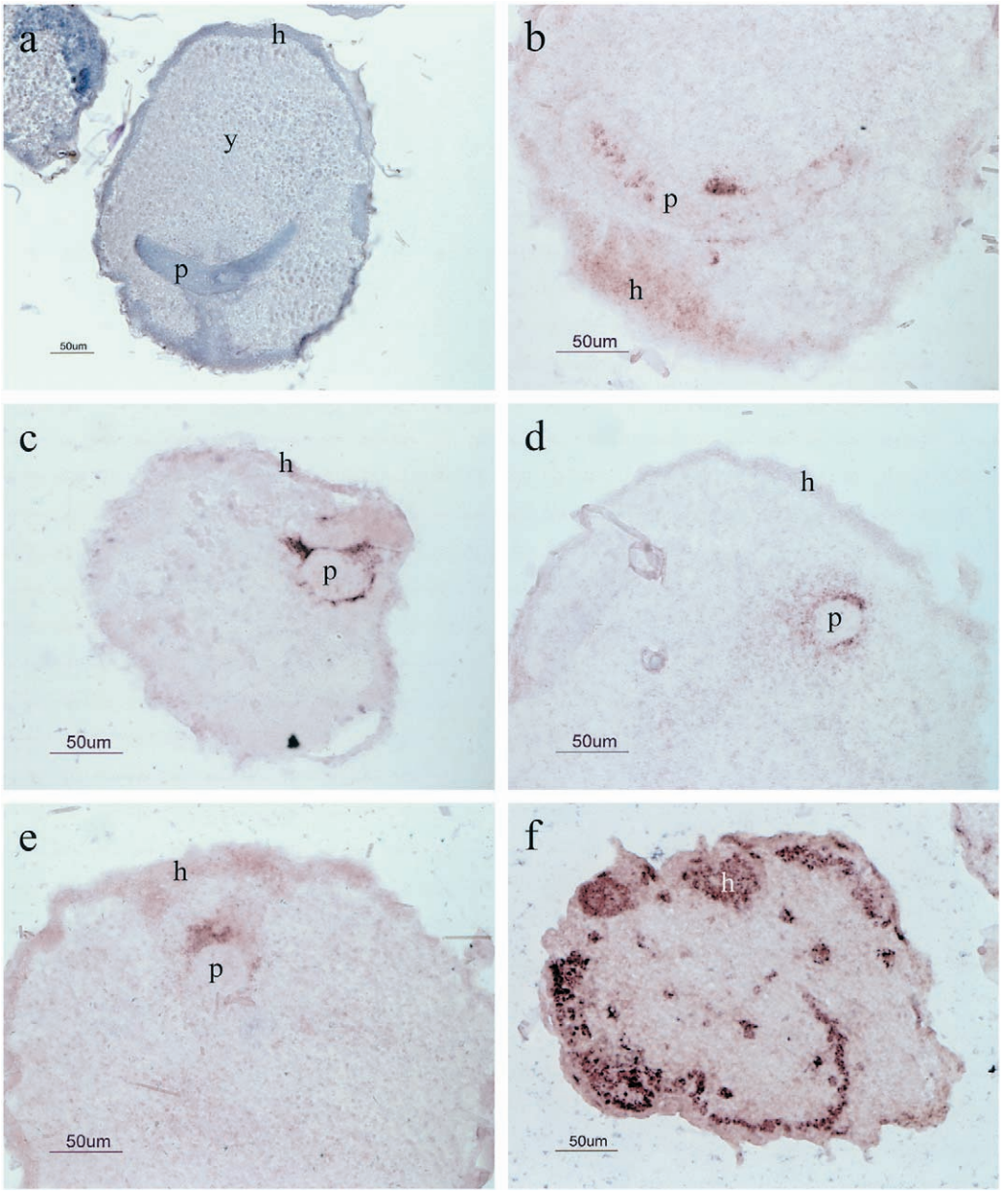

Fig. 2. HE staining (a) and in situ hybridization (b–f) of *S. littoralis* eggs 5 h after parasitization. The host embryo is always visible in the periphery of the egg. (a) and (b) are consecutive sections and show a longitudinal section through the parasitoid egg. In (c–e) cross sections of the parasitoid egg are visible and in (f) the parasitoid egg is not visible. Twenty-seven to thirty-two hour old eggs were used for parasitization with the exception of (c) where 5–8 h old eggs were used. Sections were probed with a CiV12 probe (b, c, d and f) or a CiV16.8 probe (e). Staining intensity was variable and does not directly reflect the amount of viral DNA. p, parasitoid egg; h, host embryo.

reached. This would mean that the period of time during which virions exist in the host is dependent on the time point of parasitization. *C. inanitus* successfully parasitizes eggs at all stages of development (Grossniklaus-Bürgin et al., 1994). After parasitization of young yolk-rich eggs virions would then exist longer than after parasitization of old eggs with fully developed embryos where virions would immediately enter host tissues. Electron microscopic investigations will have to be done to clarify this point. In hosts of larval parasitoids, virions were seen to enter host tissues within 1–2 h after parasitization and nucleocapsids appear to pass through nuclear pores (Stoltz and Vinson, 1977, 1979; De Buron and Beckage, 1992; Strand, 1994). Twenty-four hours after parasitization no virus-related structures were seen (Stoltz and Vinson, 1979).

The absolute quantity of viral DNA found in fifth instar hosts was 440 ± 145 pg (Table 1). Wasps were shown to inject approximately 120 pg of viral DNA along with one egg and there was a correlation with the degree of superparasitism (Lanzrein et al., 2001). For the DNA dot blot analyses we used slightly superparasitized

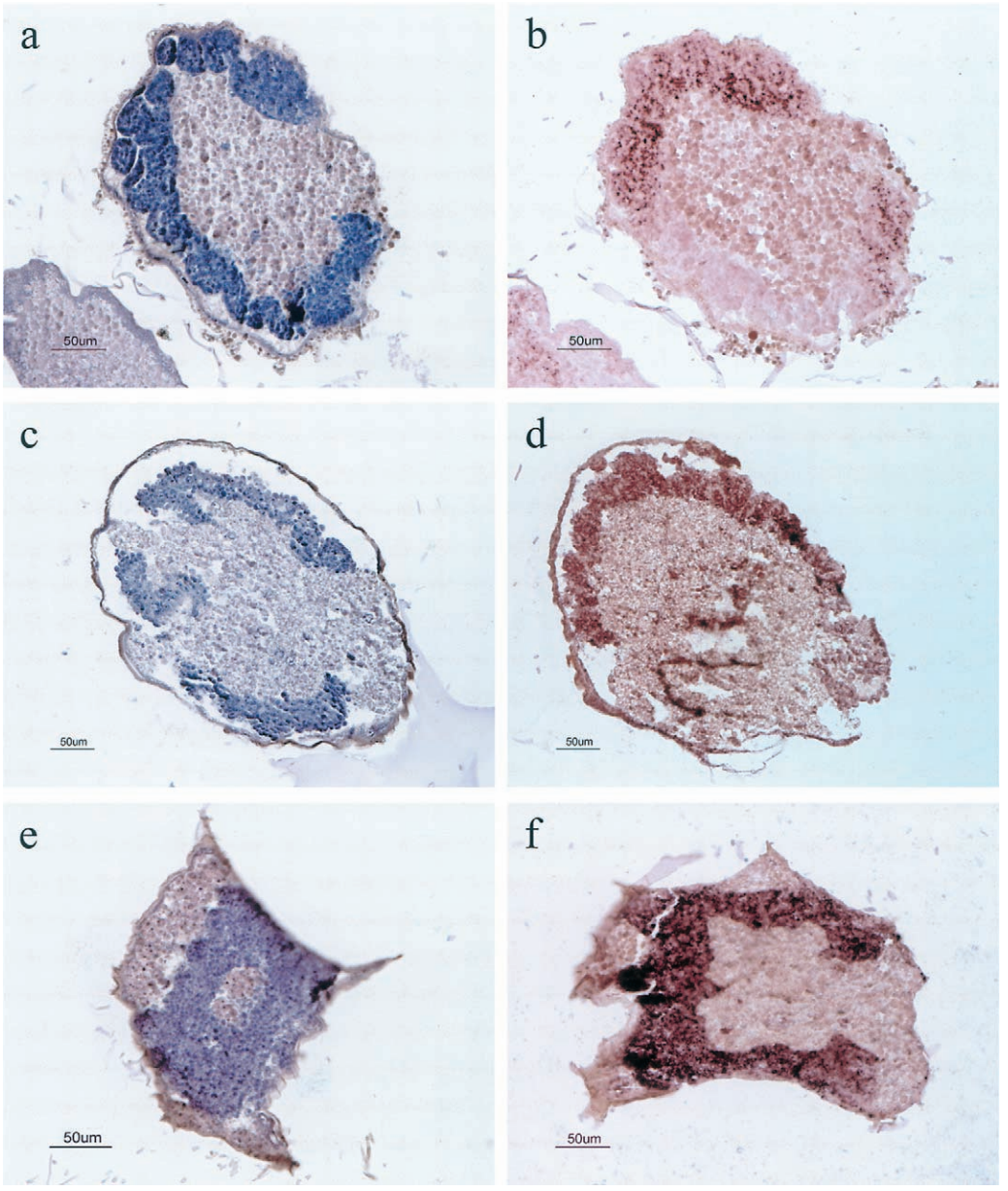

Fig. 3. HE stained sections (a, c, e) and corresponding consecutive sections (b, d, f) after in situ hybridization. Twenty-seven to thirty-two hour old eggs were parasitized and analysed 10 h (a, b) or 15 h (c–f) after parasitization. A CiV16.8 probe was used in (b) and (d) and a CiV12 probe was used in (f).

larvae, and in this case the amount of viral DNA can go up to 500 pg (Lanzrein et al., 2001). Thus, the viral DNA appears to persist in the host in the course of parasitization. Persistence of viral DNA was also seen in two hosts of larval parasitoids (Theilmann and Summers, 1986; Strand et al., 1992). Haemocytes, fat body, nervous tissue, intestinal tract and carcass all contained viral DNA (Table 1, Fig. 5). Of the three viral segments tested, all were found in all tissues and relative quantities were only slightly different (Fig. 5).

Relative to the quantity of host DNA, the concentration of viral DNA was, by far, the highest in haemocytes, about five times lower in fat body and nervous tissue and much lower in intestinal tract (Table 1). In *Pseudoplusia includens* parasitized by *Microplitis demolitor*, by far the highest amount of viral DNA relative to the tested host DNA was seen in haemocytes, only traces were seen in nervous tissue and nothing was detected in gut and fat body by 24 h after parasitization (Strand et al., 1992). In *Pseudaletia separata* injected with polydnavirus/venom of *Cotesia kariyai* highest quantities of viral DNA relative to the tested host DNA were seen in haemocytes, somewhat less in fat body and even less in nervous tissue by 48 h after injection (Hayakawa et al., 1994). By electron microscopic investigations it has been shown that fat body, muscle, hae-

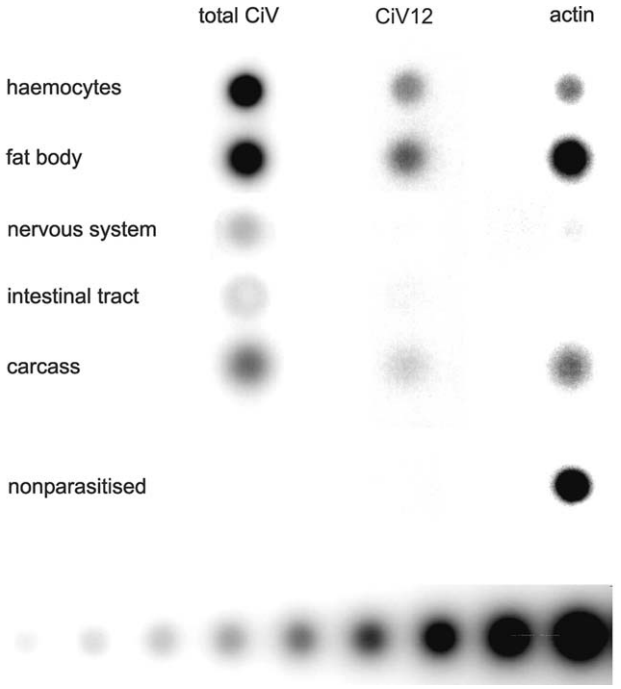

Fig. 4. DNA dot blot with various tissues of fifth instar parasitized *S. littoralis* larvae. DNA of tissues of five larvae (haemocytes, 1 μg; nervous tissue and intestinal tract, 4 μg each; fat body and carcass, 10 μg each) was dotted onto the membrane and probed first with total CiV and, after stripping, with CiV12 and actin. As a negative control, DNA of half of a nonparasitized fifth instar larva (10 μg DNA) was used. On the bottom, a CiV DNA dilution series (nine dots, steps of 1:2 from 5 to 19.5 pg) probed with total CiV is shown.

Table 1
Amounts and distribution of CiV DNA in various tissues of parasitized fifth instar *S. littoralis* larvae in feeding phase and in the parasitoid larva (late L1 and early L2)

		CiV DNA (pg ± SD)	CiV DNA/host DNA (pg/μg)
Host tissues	Haemocytes	133 ± 87	499
	Fat body	187 ± 93	102
	Nervous system	48 ± 15	95
	Intestinal tract	19 ± 15	18
	Carcass	57 ± 49	51
Sum		444 ± 145	
Parasitoid larva		820 ± 80	

Values are means ± SD per animal equivalent of 5–7 independent determinations for host tissues and of three independent determinations for parasitoid larvae. The viral content of haemocytes was multiplied with a correction factor as the amount of haemolymph collected with our method was determined to yield only 75 ± 6% (mean of four measurements with 5–9 larvae each) of total haemolymph volume as measured with inulin (Steiner et al., 1999).

mocytes, tracheae, midgut, perineurium, epidermis and Malpghian tubules are all invaded by polydnaviruses (Stoltz and Vinson, 1977, 1979; De Buron and Beckage, 1992). Thus, there is no indication of a qualitatively selective infection of particular tissues by polydnaviruses; from a quantitative perspective, however, haemocytes appear to harbour the highest load of viral DNA. In hosts of several larval parasitoids haemocytes

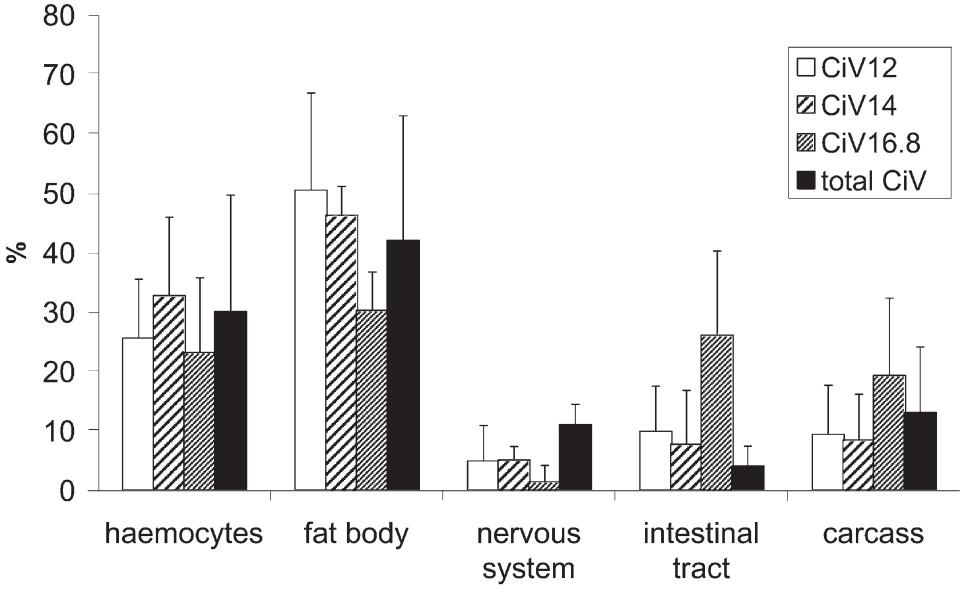

Fig. 5. Tissue distribution in percentage of total CiV DNA and of CiV12, CiV14 and CiV16.8 in fifth instar parasitized *S. littoralis* as determined by DNA dot blot. Dots were quantified on a PhosphorImager and data are means ± SD of 5–7 independent determinations for total CiV and of 2–4 independent determinations for the various segments.

have been shown to be a major site of expression of viral genes and for some a role in suppression of the host's immune system has been documented; furthermore, expression levels were highest shortly after parasitization (reviewed in Webb, 1998; Schmidt et al., 2001). In contrast, the expression of viral genes in the host of the egg–larval parasitoid *C. inanitus* is low in the early phase of parasitism and increases in later stages (Johner et al., 1999; Johner and Lanzrein, 2002). Interestingly, spreading behaviour and ultrastructure of host haemocytes are not affected by polydnaviruses in this system (Stettler et al., 1998), although they contain the highest amount of viral DNA (Table 1) and are a major site of viral gene expression (Johner et al., 1999, Blank, unpublished). This suggests that viral genes expressed in host haemocytes are not necessarily linked with changes in haemocyte behaviour and ultrastructure.

The quantity of viral DNA injected by the two larval parasitoids investigated up to now is 6.5 and 2.4 ng (Theilmann and Summers, 1986; Strand et al., 1992). This is more than the 0.1–0.4 ng injected by the egg–larval parasitoid *C. inanitus* (Lanzrein et al., 2001). *C. inanitus* is a rather large parasitoid (Grossniklaus-Bürgin et al., 1994), while the amount of viral DNA injected is comparatively low; thus the relative abundance of circular viral DNA appears to be much higher in hosts of larval parasitoids. This may have to do with the fact that in these hosts, high expression of viral genes is required immediately after parasitization to suppress the host's immune system. In the parasitoid larvae dissected out of the fifth instar hosts, 820 ± 80 pg of viral DNA were detected (Table 1). At this stage, the viral DNA is integrated in the parasitoid's genome (Gruber et al., 1996; Wyder et al., 2002). The comparison with the 440 ± 145 pg viral DNA found in host tissues in excised circular form (Table 1) reveals that the amount of viral DNA injected in virions represents about one third of the total viral genetic information present in the host at this late stage of parasitism. It will have to be analysed in future experiments to which extent expression of viral genes is related to the quantity of viral DNA in various tissues.

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