**INTRODUCTION**

In Brazil, *Anticarsia gemmatalis* multicapsid nucleopolyhedrovirus (AgMNPV) has been applied for several decades to control the cassava pest, *A. gemmatalis* (Moscardi, 1999). AgMNPV is considered to be a model for baculovirus-based biocontrol, with annual coverage now exceeding 2 million hectares. Recently, we published a sequence analysis of the 12.5 kbp BamHI D restriction endonuclease fragment from the AgMNPV-2D genome (GenBank accession no. AY542374; Slack et al., 2004). We noted several variations in this region of AgMNPV compared with other group I nucleopolyhedroviruses (NPVs), including the absence of the *v-cath* and *ChiA* genes in the gp64 locus. We also noted that AgMNPV shared two gene homologues with *Choristoneura fumiferana* multicapsid nucleopolyhedrovirus (CiMNPV) that were not found in most other baculoviruses: *v-trex* (CiMNPV ORF 113; GenBank accession no. NP_848425.1) and *p22.2CF* (CiMNPV ORF 126; GenBank accession no. NP_848438.1). The gene *v-trex* (viral three-prime repair exonuclease) was named due to its homology with a group of mammalian exonucleases (TREXs) that were described recently by Mazur & Perrino (1999, 2001). Type III exonucleases have been shown to participate in the protection of *Escherichia coli* from UV light inactivation (Serafini & Schellhorn, 1999). Baculoviruses are susceptible to inactivation by UV light (Shapiro et al., 2002) and it is important to study the *v-trex* gene from this standpoint.

In the following investigation, we sought to determine whether the AgMNPV *v-trex* gene was expressed and whether the V-TREX protein product functions as a 3’ to 5’ exonuclease. RT-PCR was used to detect *v-trex* transcripts in the context of AgMNPV infection. The AgMNPV *v-trex* ORF was also cloned into the baculovirus *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) under the control of a polyhedrin (*polh*) promoter. A fluorescence-based assay was then used to examine the exonuclease activity of the overproduced V-TREX protein.

**METHODS**

**Cell lines and viruses.** *Spodoptera frugiperda* (SF-9) cells were used to propagate wild-type (wt) AcMNPV (strain E2), BacPAK6 (Clontech) and recombinant BacPAK6 viruses. For this study, BacPAK6 is referred to as BacPAK-β-gal, with reference to the β-galactosidase gene that replaces the *polh* gene in this virus (Kitts & Possee, 1993). *A. gemmatalis*-derived UFL-Ag-286 cells (Ag-286) (Sieburth & Maruniak, 1988) were used to propagate wt AgMNPV-2D virus. Cell lines were cultured at 27°C in TNM-FH medium (Hink, 1970) containing 10% (v/v) fetal bovine serum.

**Constructs and recombinant baculoviruses.** The 693 bp ORF of *v-trex* was amplified from AgMNPV genomic DNA by PCR and DNA recombination has been proposed (Mazur & Perrino, 1999, 2001). Type III exonucleases have been shown to function independently from the DNA replication complex. A biological role for mammalian TREX proteins has not been defined, but involvement in DNA mismatch repair, DNA UV damage repair and...
cloned into the baculovirus transfer vector plasmid p BacPAK8 (Clontech). The PCR primers TREX-LP-XhoI-NcoI (5′-AACATCTAGGGTCCCATGACTGTTGAAGACGTTTG-3′) and TREX-RP-NcoI-NcoI del (5′-TTATAACGATGGCCGCTCATGCCGAGGATGACGTTTG-3′) were used to engineer 5′ XhoI and 3′ NcoI restriction sites on the ends of the v-trex ORF such that it could be cloned downstream of the polH promoter of the pBacPAK8 plasmid. The resulting construct, pBacPAK8-v-trex, was co-transfected with Bsu36I-digested BacPAK6 viral DNA into Sf-9 cells, as described by Kitts & Possee (1993). Transfections were facilitated by using the lipid transfection agent Cellfectin (Invitrogen). BacPAK-v-trex virus clones were isolated by plaque purification.

Preparing cell lysates for TREX assays. T-75 tissue-culture flasks of Sf-9 cells (1 × 10⁷ cells per flask) were infected at an m.o.i. of 1. At 72 h post-infection (p.i.), cells were collected in 50 ml Corning tubes and counted. Cells were pelleted at 1000 g for 1 min and suspended in 25 ml chilled PBS/EDTA (125 mM NaCl, 10 mM Na₂HPO₄, 5 mM EDTA, 2-5 mM KCl, pH 6-2). Cells were pelleted again at 1000 g for 1 min and suspended in 5 ml chilled PBS/EDTA. Finally, cells were pelleted at 1000 g and suspended at a concentration of 5 × 10⁶ cells ml⁻¹ in TREX dilution buffer [75 mM NaCl, 50 mM Tris/HCl (pH 8-0), 5 mM Na₂HPO₄, 2-5 mM EDTA, 2 mM DTT, 5% (v/v) glycerol, 2% (v/v) ethanol, 0-25 mM Ac-Leu-Leu- norleucinal (cysteine protease inhibitor)]. Suspended cells were frozen overnight at −20 °C and then thawed on ice and disrupted by sonication for 30 s using a Microson XL ultrasonic cell disruptor (Heat Systems). Cell lysates were centrifuged at 4500 g for 5 min at 10 °C. Supernatants were collected into 1-5 ml Eppendorf tubes and assayed for total protein by using a Coomassie Plus Protein Assay kit (Pierce). Supernatants were diluted in TREX dilution buffer to a protein concentration of 1 mg ml⁻¹. The resulting soluble lysates were used in exonuclease assays.

Budded virus (BV) purification and processing. Cell-culture supernatant volumes of 33 ml from 4 × 10⁶ infected Ag-286 cells were collected at 52 h p.i. Cellular debris was removed by centrifuging twice at 1000 g for 5 min. BV-containing cell-culture supernatants were centrifuged for 1-5 h at 100 000 g at 10 °C through a 5 ml cushion of 20% (w/w) sucrose in PBS (pH 7-4) containing 5 mM iodoacetamide (cysteine protease inhibitor), 5 mM EDTA. BV pellets were suspended in 300 μl TREX dilution buffer and frozen at −20 °C. BV samples were thawed on ice and sonicated for 15 s. BV lysates were assayed for total protein by using a Coomassie Plus Protein Assay kit. BV lysates were diluted in TREX dilution buffer to a protein concentration of 0-9 mg ml⁻¹.

Exonuclease assays. Exonuclease assays were done in 96-well U-bottomed plates. Plates were placed on ice while reagents were combined. Lysate volumes of 10 μl were combined with 30 μl TREX assay buffer [20 mM Tris/HCl (pH 7-5), 5 mM MgCl₂, 2 mM DTT, 100 μg BSA ml⁻¹]. During assays, plates were covered with aluminum foil and incubated for 1 h at 37 °C. Assays were stopped with 20 μl TREX stop buffer [50% (v/v) formamide, 3 × TBE, 15% (w/v) sucrose] and assays were stored at 4 °C until analysis. All TBE solutions were made from a 10 × TBE stock (890 mM H₂BO₃, 450 mM Tris-base, 20 mM EDTA, pH 8-0). Exonuclease assay sample volumes of 20 μl were fractionated by electrophoresis (3 h, 25 mA, 200–300 V) in 13% (v/v) acrylamide:N,N'-methylene-bis-acrylamide (20:1), 1 × TBE, 5 M urea gels. Electrophoresis was done by using a Hoeffer SE600 vertical gel unit and 0-7 mm × 18 cm × 16 cm gels. Gels were scanned in their plates by using a Typhoon fluorescent scanner (Amersham Biosciences) that had been set to 3 mm above the focal plane. For exonuclease assays, two 35 nt, fluorescently labelled DNA oligomers were synthesized at a 25 μmol scale (Integrated DNA Technologies). One oligomer (5'HEX-oligo) was covalently linked at its 5’ end to hexamethylfluorescein (5’-HEX-GTCAGACACTCCGCAAGCCTTAGCTACATTCCACACATC-3’).

The other oligomer (3FAM-oligo) was covalently linked at its 3’ end to 6-carboxymethylfluorescein (5’-AGGACACATGACTGGGTCAGGCGGATGAGC-3’). In some experiments, the 5HEX-oligo and the 3FAM-oligo partially annealed to each other such that 25 nt annealed, leaving 10 mismatched nucleotides on the non-labelled ends that did not anneal (see Fig. 7a). Assays containing the 5HEX-oligo were scanned at excitation 532 nm/emission 555 nm BP 20 nm. Assays containing the 3FAM-oligo were scanned at excitation 532 nm/emission 526 nm SP. Assays containing both oligomers were scanned at dual wavelengths and images were separated by using Fluorosep 2.2 software (Amersham Biosciences). All Typhoon-scanned images were analysed on ImageQuant 5.0 (Amersham Biosciences).

RNA preparation and RT-PCR. T-75 tissue flasks were seeded with 5 × 10⁶ Ag-286 cells and infected at an m.o.i. of 10 with 4-5 ml viral inoculate. After 2 h rocking, the medium was removed and replaced with 10 ml fresh medium. At various times, cells were harvested by suspension in medium. Cells were pelleted at 1000 g for 1 min and suspended in 11 ml chilled PBS/EDTA. Cells were pelleted at 1000 g for 1 min and processed for total RNA by using an RNAsesy Mini kit (Qiagen). RNA samples (100 μl) were stored at −20 °C. Aliquots of 5 μl RNA (500 ng) were used in 100 μl PCRs or RT-PCRs. To eliminate DNA contamination, 5 μl RNA samples were combined with 5 μl 2 × restriction endonuclease buffer no. 3 (New England Biolabs) containing the enzymes D Nas I (30 μl), Nco I (150 μl) and Mlu I (150 μl). Mlu I and Nco I are restriction enzymes that cut the v-trex gene (Fig. 1). After 1 h at 37 °C, 1 μl 25 mM EDTA was added and enzymes were heat-inactivated for 30 min at 65 °C. RT-PCRs and PCRs were then done by using an Access RT-PCR kit (Promega). The D Nase/restriction enzyme-treated RNA samples (11 μl) were combined with 100 μl 1 × reaction buffer (RB; Promega) containing 1-3 mM MgSO₄ and 200 μM dNTPs. The resultant RNA solution was split into two 0-5 ml Eppendorf tubes. One tube (RT-PCR) received 1 μl (5 U) avian myeloblastosis virus reverse transcriptase and the other control tube (PCR) received no enzyme. The v-trex gene antisense primer TREX-RP (5’-ATATGTAAGCTTTTCCCCCATAGGGATGACGTTTG-3’; Fig. 1) was added (50 pmol per tube). Both RT-PCR and PCR tubes were incubated at 48 °C for 1 h and then chilled on ice. A 5 μl aliquot of 1 × RB containing 50 pmol of the sense primer TREX-LP-XhoI-NcoI and 5 U Tfl I DNA polymerase was added. Tubes were sealed with 50 μl mineral oil and placed in a 95 °C pre-heated block of a Perkin-Elmer-Cetus thermocycler for 2 min. The PCR was run for 60 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 47 °C and extension for 1 min at 68 °C.

RESULTS AND DISCUSSION

This study investigated a recently described baculovirus gene, v-trex (Slack et al., 2004). Our aim was to provide evidence that the v-trex gene is expressed by AgMNPV and that the V-TREX protein has exonuclease activity that would confirm the nomenclature.

Evidence for v-trex expression

From the AgMNPV DNA sequence, it was predicted that the v-trex gene would be expressed at early times during infection. This prediction was based on the presence of an ATCAGT motif 7 bp upstream of the v-trex translation start point (Fig. 1). In addition, the v-trex gene promoter region has a TATAA box and the eukaryotic transcription factor-binding motifs CACGTG and GATA. These elements have been shown to be important for the transcription of
early baculovirus genes (Kogan & Blissard, 1994; Shippam-Brett et al., 2001). The GATA element may not be ideal, as it overlaps the TATAA box. With RT-PCR, we were able to detect the presence of v-trex RNA transcripts from 3 to 72 h p.i (Fig. 2). This result confirmed the prediction that v-trex is an early gene. There were no late promoter (A/T)TAAG motifs in the vicinity of v-trex. The presence of v-trex RNA transcripts late in infection may be the result of v-trex transcript stability. The end of the v-trex gene contains a strong polyadenylation signal motif, ATAATAAA, which would promote the production of more stable, polyadenylated transcripts.

Fig. 1. Sequence of the AgMNPV v-trex gene region. The v-trex gene lies between the AgMNPV homologues for Lef-7 and AcMNPV ORF 124 (ORF124Ac). DNA sequence motifs are indicated in upper case: CACGTG and GATATAA sequences in the v-trex promoter region and an ATAATAAA polyadenylation signal at the end of the v-trex gene. The translational start point (tsp) of the v-trex gene is indicated by an arrowhead. Numbering on the right-hand side indicates nucleotide positions relative to the v-trex tsp. Restriction sites for MluI and Ncol that were used to eliminate DNA background from RT-PCRs are labelled and boxed. The location of the primer TREX-RP that was used to generate the initial reverse-transcribed DNA strand in the RT-PCR is also indicated. The protein amino acid sequence is written below the DNA sequence. For V-TREX, the amino acid residues of the exonuclease motifs ExoI, ExoII and ExoIII are shown in bold type. Conserved active-site residues are underlined.

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Fig. 2. RT-PCR detection of v-trex gene transcripts. RNA was purified from control uninfected Ag-286 cells (M) and from Ag-286 cells that had been infected with AgMNPV for 3, 6, 8, 24 or 72 h. A portion of each RNA purification product was used for PCR and RT-PCR to detect v-trex gene transcripts. PCR and RT-PCR products were fractionated on a 1% 25% (w/v) agarose/1 X TAE gel. A 1 kb Plus Ladder (Invitrogen) DNA size standard (STD) was also run. The gel was stained with ethidium bromide and visualized by using a Typhoon fluorescence imager (532 nm excitation/emission 610 nm BP 30).
Overexpression of \( v\)-trex

To characterize the V-TREX protein, we overexpressed the \( v\)-trex ORF in an AcMNPV-based expression vector system (AcMNPV does not have a \( v\)-trex homologue). The recombinant AcMNPV virus was called BacPAK-\( v\)-trex and placed the \( v\)-trex ORF under the control of a polh promoter. The recombinant virus produced an abundant protein with an estimated size of 23.7 kDa that represented 8% of total soluble protein in lysates from BacPAK-\( v\)-trex-infected Sf-9 cells. This protein was not present in the parent BacPAK-\( \beta\)-gal virus, wt AcMNPV or in Sf-9 cells (Fig. 3). We concluded from this that the V-TREX protein was overproduced successfully by the BacPAK-\( v\)-trex virus. With an apparent molecular mass of 23.7 kDa, V-TREX migrated faster in SDS-PAGE than its predicted size of 25.4 kDa. V-TREX has no strong predictions for post-translational modifications that might affect migration. However, V-TREX has an estimated isoelectric pH of 8.0, 4.5, 1.5, 0.5, 0.17, 0.06, 0.02.

Fig. 3. SDS-PAGE analysis of proteins produced by BacPAK-\( v\)-trex virus. Soluble protein lysates from control uninfected Sf-9 cells (lane M) and Sf-9 cells that had been infected with BacPAK-\( v\)-trex (lane 1), BacPAK-\( \beta\)-gal (lane 2) or wt AcMNPV (lane 3) were denatured in SDS-PAGE disruption buffer (250 mM Tris/HCl, 2% SDS, 5% mercaptoethanol, 20% glycerol, 0.4% bromophenol blue) and fractionated by 12% acrylamide : N,N'-methylene-bis-acrylamide (37 : 1) SDS-PAGE. A Precision Plus protein standard (Bio-Rad) was run alongside samples; protein sizes are indicated on the left. The gel was stained with Coomassie brilliant blue.

Fig. 4. Exonuclease activity assay of the BacPAK-\( v\)-trex virus. Soluble protein lysates from control uninfected Sf-9 cells and Sf-9 cells that had been infected with wt AcMNPV, BacPAK-\( \beta\)-gal or BacPAK-\( v\)-trex were assayed for exonuclease activity (see Methods). A one-third dilution series was made of each lysate group in TREX dilution buffer. Exonuclease reactions were fractionated by electrophoresis in a denaturing 13% acrylamide/urea gel. Total amount (ng) of protein lysate used in each exonuclease reaction is indicated above each lane.
which would give this protein a net positive charge in SDS-PAGE buffer at pH 6.8.

**Detection of V-TREX-specific 3’ to 5’ exonuclease activity**

We next determined whether the high levels of V-TREX production by the BacPAK-v-trex virus corresponded to increased levels of 3’ to 5’ exonuclease activity. We developed a fluorescence-based exonuclease assay to replace the conventional radioisotope-based assay. Two fluorescently labelled DNA oligomers were used as substrates in exonuclease assays: 5HEX-oligo and 3FAM-oligo. The 5’-labelled 5HEX-oligo was used for most experiments.

Soluble protein lysates from insect cells were diluted serially and incubated at 37°C with the 5’-fluorescently labelled 5HEX-oligo. Exonuclease assays included lysates from control uninfected Sf-9 cells and Sf-9 cells that had been infected with wt AcMNPV, BacPAK-β-gal or BacPAK-v-trex. Exonuclease assays were analysed in denaturing acrylamide/urea gels. Lysates from Sf-9 cells produced a

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**Fig. 5.** Effect of unlabelled competitor, EDTA and divalent cations on V-TREX exonuclease activity. Soluble protein lysates from Sf-9 cells that had been infected with BacPAK-v-trex or BacPAK-β-gal were diluted in TREX dilution buffer to give 80 ng lysate protein per exonuclease assay. Exonuclease reactions were fractionated by electrophoresis in a denaturing 13% acrylamide/urea gel. (a) Unlabelled oligomers were added at increasing concentrations to compete with 0.25 mM 5HEX-oligo. Concentration (mM) of unlabelled competitor oligomers in each reaction is indicated above each lane. (b) Increasing concentrations (mM) of EDTA were added to the exonuclease assays as indicated. These exonuclease assays also included 5 mM MgCl₂. (c) Increasing concentrations (μM) of different divalent cations (in the form of MgCl₂, MnCl₂, ZnCl₂ and CaCl₂) were added to the exonuclease assays as indicated. The type of lysate present is indicated at the bottom: BacPAK-v-trex (V-TREX or VT) or BacPAK-β-gal (β).
gradient of faster-migrating 5HEX-oligonucleotides when
protein amounts were 1100–3300 ng (Fig. 4, top panel).
Lysates from wt AcMNPV and BacPAK-β-gal produced
faster-migrating 5HEX-oligonucleotides when protein
amounts were 370–3300 ng (Fig. 4, middle panels). Our
interpretation of these results was that the increased
mobility of 5HEX-oligo was the result of exonuclease
activity decreasing the size of the oligomers. Lysates from
BacPAK-v-trex caused the 5HEX-oligo to shift to a faster-
migrating species when protein amounts were 45–3300 ng
(Fig. 4, bottom panel). At protein amounts between 0.17
and 1.5 ng, there was a gradient of 5HEX-oligo sizes.

We estimated that the BacPAK-v-trex virus lysates contained
exonuclease activity that was 6000-fold greater than the
activity in lysates from uninfected Sf-9 cells. In addition,
BacPAK-v-trex lysates had at least 2000-fold greater activity
than lysates from BacPAK-β-gal or wt AcMNPV-
infected Sf-9 cells. We concluded that the greatly increased
exonuclease activity associated with the BacPAK-v-trex
virus was due to the presence of V-TREX. The large increase
in exonuclease activity suggested that V-TREX does not
require the proportional presence of other viral proteins
for activity. This supports the prediction that V-TREX is an
independently active exonuclease (Slack et al., 2004).

Effects of pH, oligomer competitors, EDTA and
divalent cations on V-TREX

V-TREX-associated exonuclease activity on the 5HEX-oligo
substrate was inhibited when unlabelled oligomers were
added in molar excess (Fig. 5a). Activity was also inhibited
in the presence of EDTA (Fig. 5b), indicating that V-TREX
is a metalloenzyme that requires the presence of divalent
cations.

The effect of different divalent cations on exonuclease
activity was examined. Initially, we screened Mg2+, Mn2+, Zn2+, Ca2+ and Cu2+ over concentration ranges of 1–50 mM. Exonuclease activity occurred in the presence of Mg2+ at all concentrations tested (data not shown). We observed residual exonuclease activity in the presence of 1 mM Mn2+. Other cations did not catalyse any exo-
nuclease activity. We carried out further exonuclease assays
with Mg2+, Mn2+, Zn2+ and Ca2+ at concentration ranges of
60–2000 μM. At this lower concentration range, it was
possible to titrate out Mg2+-catalysed V-TREX exonuclease
activity to 250 μM (Fig. 5c). It was surprising to observe that
Mn2+ catalysed some exonuclease activity between
250 and 500 μM. A similar window of Mn2+-catalysed
exonuclease activity has been reported for the baculovirus
alkaline nuclease (AN) protein (Li & Rohrmann, 2000). In
that study, Mn2+-catalysed AN activity was lower than
Mg2+-catalysed activity. It was later reported that Mn2+
produced lower levels of AN activity, but did not inhibit
activity (Mikhailov et al., 2004). In the present study,
5HEX-oligos were only partially digested in the presence of
Mn2+, compared with complete digestion in the presen-
ence of Mg2+. Based on the homology of V-TREX with
mammalian TREX proteins (Mazur & Perrino, 2001), it
had been predicted that V-TREX would be activated to
similar levels in the presence of either Mn2+ or Mg2+. There
may a physiological difference between mammalian cells
and baculovirus-infected insect cells that has driven the
evolution of V-TREX to become more selective towards
Mg2+.

Exonuclease assays were carried out over a range of pH
values (Fig. 6). V-TREX activity was optimal between
pH 6.1 and 7.4. This differentiates V-TREX from the
more alkaline-active baculovirus exonuclease AN (Li &
Rohrmann, 2000). V-TREX also has a more acidic activity
profile than mammalian TREX proteins (Mazur & Perrino,
2001).

V-TREX activity on 3′-labelled oligomers and
dsDNA

Further exonuclease experiments were done with different
oligomer substrate combinations that included the 3′-
labelled 3FAM-oligo (Fig. 7a). In this set of exonuclease
experiments, only residual exonuclease activity was detected
in cell lysates from BacPAK6-β-gal virus-infected Sf-9 cells
(Fig. 7c). We assumed that the exonuclease activity that

Fig. 6. Effect of pH on V-TREX exonuclease activity. Soluble
protein lysates from Sf-9 cells that had been infected with
BacPAK-v-trex or BacPAK-β-gal were diluted in TREX dilution
buffer to a concentration of 10 ng lysate protein per exo-
nuclease assay. Extract volumes of 5 μl were combined with
45 μl pH-adjusted buffer (5 mM MgCl2, 50 mM Tris/HCl,
50 mM Na2CO3, 2 mM DTT, 100 μg BSA ml−1). Exonuclease
reactions were fractionated by electrophoresis in a denaturing
13% acrylamide/urea gel.
was detected in cell lysates from BacPAK-\(v\)-trex virus-infected Sf-9 cells was mostly V-TREX activity (Fig. 7b).

V-TREX produced different results when acting on 5' labelled and 3' labelled ssDNA substrates. As in earlier assays, increasing amounts of V-TREX extracts generated a gradient of smaller 5HEX-oligo fragments (Fig. 7b, panel 1). In contrast, the 3FAM-oligo abruptly dropped to a very small size when treated with V-TREX extracts (Fig. 7b, panel 3). This was interpreted to be the result of V-TREX cleaving off the labelled terminal nucleotide on the 3' end of the 3FAM-oligo. These results are as would be predicted for a 3' to 5' exonuclease and are the converse of what others have observed for the baculovirus 5' to 3' exonuclease AN (Mikhailov et al., 2003).

To examine the effects of dsDNA on V-TREX exonuclease activity, the 5HEX-oligo and 3FAM-oligo were annealed. The HEX and FAM fluorescent labels could be seen separately in the same gel, due to different emission spectra.
(see Methods). The annealed 5HEX-oligo and 3FAM-oligo substrates required more TREX extract in order to be digested (Fig. 7b, panels 2 and 4). This indicated that V-TREX exonuclease activity has some ssDNA specificity. The 5HEX-oligo and 3FAM-oligo design was such that when these 35 nt oligomers were annealed, 10 bp mismatched ends would be present (Fig. 7a, 5H/3F). An intermediate-sized 5HEX-oligo product was generated at protein extract concentrations of 41–123 ng (Fig. 7b, panel 2). No such intermediate-sized products were generated from the 3FAM-oligo (Fig. 7b, panel 4). V-TREX thus exhibited characteristics of a 3′ to 5′ repair exonuclease by targeting the misannealed 3′ end. This type of activity has been observed for mammalian TREX proteins (Mazur & Perrino, 2001).

**Exonuclease activity associated with AgMNPV BV**

Experiments were done using the 5′-labelled 5HEX-oligo to determine whether there was 3′ to 5′ exonuclease activity associated with AgMNPV infection. Soluble protein lysates from Ag-286 cells that had been infected with AgMNPV were compared with lysates from uninfected Ag-286 cells. The relative amount of 3′ to 5′ exonuclease activity associated with AgMNPV-infected Ag-286 cells was not significantly different from that of uninfected Ag-286 cells (data not shown).

We also looked for exonuclease activity associated with AgMNPV BV. Ag-286 cells were infected with AgMNPV or AcMNPV. It was ensured that similar levels of infection had been achieved (Fig. 8a) and that sucrose-cushion ultracentrifugation-purified virion preparations were diluted to contain similar amounts of total protein (Fig. 8b). The 5HEX-oligo substrate was incubated with sonicated BV preparations from AgMNPV and AcMNPV. Significantly more exonuclease activity was present in AgMNPV BVs than in AcMNPV BVs (Fig. 8c).

Insect cells already contain significant levels of 3′ to 5′ exonuclease activity such that wt AgMNPV-infected cells do not have detectably elevated exonuclease levels due to V-TREX. Our data point to the possibility that V-TREX is specialized to associate with BVs of AgMNPV. However, we acknowledge that the present data are inconclusive and that more direct evidence is needed to confirm that V-TREX is the origin of 3′ to 5′ exonuclease activity in AgMNPV BVs.

**Conclusions**

The evidence presented in this study leads to the conclusion that the v-trex gene product is a functional 3′ to 5′ exonuclease and that V-TREX belongs to the TREX family of exonucleases. At 23–7 kDa, V-TREX is one of the smallest functional 3′ to 5′ exonucleases to be described. V-TREX showed remarkable stability throughout this study, with activity varying little over several months of repeated freezing and thawing. Recently, a v-trex gene homologue appeared in GenBank as ORF 119 of the C. fumiferana defective NPV (CfDEFNPV) baculovirus genome (accession no. AY327402.1). The CfDEFNPV v-trex homologue is
predicted to encode a protein that is 148 aa in size and appears to be missing one-third of its C-terminus.

The v-trex gene has not been identified in the genomes of most other sequenced baculovirus species. There are no v-trex homologues in other virus families and v-trex is most similar to eukaryotic genes. This suggests that the v-trex gene was probably acquired recently in baculovirus evolution. Studies are currently being done to determine whether the v-trex gene is essential for the replication of AgMNPV and what biological function v-trex may have. We anticipate that v-trex will be classified as a baculovirus auxiliary gene, along with such genes as v-cath (Slack et al., 1995) and ChiA (Hawtin et al., 1995).

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