Baculoviruses contain a gene for the large subunit of ribonucleotide reductase

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In the genomes of two baculoviruses, Spodoptera exigua and S. littoralis multicapsid nucleopolyhedroviruses (SeMNPV and SpliMNPV, respectively), an open reading frame (ORF) encoding the large subunit of ribonucleotide reductase (RR1) was identified. The predicted amino acid sequences of SeMNPV and SpliMNPV RR1 showed high homology to RR1 proteins from eukaryotes (ca. 70% and 80% similarity, respectively). The amino acid residues thought to be involved in catalytic function were conserved in the baculoviral RR1 ORFs. The RR1 ORFs in SeMNPV and SpliMNPV were located in different genomic positions. In SeMNPV, the RR1 ORF was located upstream of the polyhedrin gene, in an anti-genomic orientation. In SpliMNPV, the RR1 ORF preceded the p74 gene. By searching databanks, sequences homologous to the N terminus of RR1 were also detected upstream of the polyhedrin gene of three other baculoviruses, Mamestra brassicae multicapsid NPV, Panolis flammea multicapsid NPV and Orgyia pseudotsugata single nucleocapsid NPV. The baculovirus type species, Autographica californica multicapsid NPV, however, does not encode RR. A 2.7 kb transcript could be detected throughout infection with SeMNPV, classifying SeMNPV rr1 as an early gene. Primer extension analysis revealed several early and late start sites. None of the major start sites showed similarity to previously characterized baculoviral transcriptional start motifs. Phylogenetic analysis of prokaryotic, eukaryotic and viral RR1 proteins suggested that SeMNPV and SpliMNPV acquired the gene for RR1 from a eukaryotic source, but independently from each other.

Introduction

Ribonucleotide reductase (RR) is a key enzyme in the biosynthesis of deoxyribonucleotides, catalysing the reduction of ribonucleotides (for reviews see: Thelander & Reichard, 1979; Reichard 1988, 1993; Elledge et al, 1992). The RR (class I) enzyme is present in all eukaryotes and in some prokaryotes, such as E. coli grown under aerobic conditions (Reichard, 1993). The E. coli RR enzyme is considered to be the prototype and functions as a heterodimer consisting of two large (RR1) and two small (RR2) subunits encoded by different genes. The actual reduction of ribonucleotides takes place at the large subunit. The small subunit supplies the reducing capacity; it contains an active iron centre and provides electrons via a tyrosyl radical. The reduction of ribonucleotides into deoxyribonucleotides is subject to complex allosteric control exerted by binding of nucleotides to the large subunit. The overall activity level of the enzyme is regulated by the binding of ATP and dATP to the large subunit. Expression of ribonucleotide reductase genes is tightly regulated during the cell cycle (Reichard, 1988; Elledge et al., 1992).

Members from several groups of large DNA viruses, such as the poxviruses, herpesviruses, T-even bacteriophages and African swine fever virus (ASFV), encode their own RR. In the animal viruses, RR also acts as a virulence factor. Deletion usually results in an attenuated phenotype with slightly impaired growth in cultured cells and increased restrictions in tissue specificity. RR-encoding viruses can apparently circumvent the strict regulation of RR activity by the cellular enzyme, as well as bypass the compromised host regulation machinery (Reichard, 1988; Conner et al., 1994; Slabaugh et al., 1984; Child et al., 1990; Cunha & Costa, 1992; Howell et al., 1993; Heineman & Cohen, 1994; Huszar & Bacchetti, 1981;
Goldstein & Weller, 1988). RR is an often sought target in the search for antiviral, antimicrobial and cancer therapies, either suppressing enzyme activity exploited by the pathogen during infection, or inhibiting the elevated RR enzyme activity of rapidly growing cancer cells, which are no longer subject to normal cell cycle control (Conner et al., 1994; Lori et al., 1994; Reichard, 1988).

RR has not yet been described for baculoviruses, another major group of large DNA viruses. Baculoviruses are pathogenic for arthropods and have a large (80–160 kbp) circular dsDNA genome (Murphy et al., 1995). They replicate in the nucleus of the infected cell. Genes encoding RR have not been reported for the type species of the Baculoviridae, Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) (Ayres et al., 1994).

Here, we present evidence that two other members of the baculovirus family, i.e. Spodoptera exigua and S. littoralis multicapsid nucleopolyhedrovirus (SeMNPV and SpliMNPV, respectively), contain an open reading frame (ORF) with a high degree of homology to the large subunit of RR1 of eukaryotic and viral origins. Expression of the SeMNPV rr1 gene in infected insect cells was investigated by transcriptional analysis. To determine the ancestry of the two baculoviral RR1s, a phylogenetic tree was constructed using parsimony.

**Methods**

- **Virus, insects and cells.** The SeMNPV field isolate (SeMNPV/US) (Gelernter & Federici, 1986) was kindly provided as polyhedra by B. A. Federici (Department of Entomology, University of California, Riverside, USA). The polyhedra were propagated in fourth instar S. exigua larvae (Smits et al., 1988). Budded virus (BV), used in time-course infection experiments, was obtained from the supernatant of UCR-Se1 (Gelernter & Federici, 1986) or IZD-Se-2109 cells (a gift from B. Moeckel, Institute of Zoology, Technical University, Darmstadt, Germany) which had been infected with haemolymph from SeMNPV-infected fourth instar larvae. The S. exigua cell lines were maintained at 27 °C in plastic tissue-culture flasks in TNM-FH medium (Hink, 1970) supplemented with 10% foetal bovine serum. BV titres were determined by the end-point dilution method and expressed as TCID<sub>50</sub> units/ml. Cells were infected with an m.o.i. of 5 TCID<sub>50</sub> per cell.

SpliMNPV type-B isolate E15 was plaque-purified from haemolymph of field-infected S. littoralis larvae in SPC-Sl-52 cells (Mialhe et al., 1984), maintained at 27 °C in TNM-FH medium (Sigma) supplemented with 10% heat-inactivated foetal calf serum.
DNA analysis. Viral DNA was extracted from SeMNPV occlusion body derived virions purified by sucrose gradient centrifugation essentially as described by Caballero et al. (1992). Viral DNA from SpliMNPV was purified from viral occlusion bodies propagated in third instar S. littoralis larvae.

Localization and DNA sequencing of the rr1 genes. SeMNPV fragment Xbal-D, containing N-terminal sequences of the polyhedrin gene (van Strien et al., 1992), was cloned into pUC18. Subfragments upstream of the polyhedrin gene were isolated from agarose gels using the freeze–sieve method (Sambrook et al., 1989) and subcloned into plasmids pTZ19R (Promega) or pBluescriptKS(+) (Stratagene). Sequencing of SeMNPV inserts with standard sequencing and custom-designed primers (Eurogentec) was performed with Taq polymerase using the chain termination method.

SeMNPV rr1 gene DNA fragments were [α-32P]dATP-labelled and hybridized under non-stringent conditions (Sambrook et al., 1989) to a Southern blot of SpliMNPV viral DNA on Hybond-N filters (Amersham). Hybridizing SpliMNPV DNA fragments were purified from agarose gels with the Jetorb kit (Genomad), (sub)cloned into pUC19 and sequenced according to the chain termination method with standard sequencing primers using the Sequenase kit (United States Biochemical).

Sequence analysis of the rr1 genes. Sequences were analysed with the UWCGG computer programs (Devereux et al., 1984); DNA and deduced amino acid sequences were compared with the updated GenBank/EMBL, SWISSPROT and PIR databases using the FASTA program and BLAST network service (Altschul et al., 1990).

Isolation of total RNA and Northern blot hybridization. For Northern blot analysis and primer extension, total RNA was isolated from SeMNPV-infected UCR-Se1 or IZD-Se-2109 cells at various times post-infection (p.i.), as described by van Strien et al. (1992). Total RNA was denatured, electrophoresed in agarose gels and blotted onto Hybond-N nylon membrane (van Strien et al., 1992). To identify rr1 transcripts, the blot was hybridized for 16 h at 65 °C with an [α-32P]dATP-labelled rr1 specific probe. After hybridization, the filter was washed for 5 min with 2 × SSC, 0.5% SDS at 65 °C, 30 min with 2 × SSC, 0.1% SDS at 65 °C and 30 min with 0.1 × SSC, 0.1% SDS at 65 °C. The filter was exposed to Kodak XAR film.

Primer extension. To identify the transcriptional start site(s) of SeMNPV rr1, 15 ng of an oligonucleotide (5’ CAGACTATTCAAGA-GGAGG 3’), complementary to the rr1 mRNA, was labelled at the 5’ end with [α-32P]dATP by T4 polynucleotide kinase (Gibco-BRL) in 50 mM Tris–HCl pH 9.5, 10 mM MgCl2, 5 mM DTT, 5% glycerol for 45 min at 37 °C followed by heat denaturation at 90 °C for 10 min. The labelled oligonucleotide was purified on a 1 ml Sephadex G25 column. Labelled primer was added to 10 μg of total infected-cell RNA and the mixture was denatured at 90 °C for 5 min and annealed at 54 °C for 15 min. Reverse transcription was carried out at 48 °C for 1 h in a volume of 15 μl, containing 5 m.M of each of the dNTPs and 1 μl Superscript reverse transcriptase (Gibco-BRL) in a buffer supplied by the manufacturer. The reaction was stopped by addition of 5 μl ‘stop’ buffer containing 95% (v/v) formamide, 0.01% xylene cyanol and 0.01% bromophenol blue. The reaction mixture (6 μl) was analysed in a 6% denaturing polyacrylamide gel.

Accession numbers. The nucleotide sequences of the SeMNPV and SpliMNPV rr1 genes are available in the EMBL/GenBank database under accession numbers X97578 and X98924, respectively.
Baculovirus early (CAGT) and late (TAAG) consensus transcriptional start sites (Blissard & Rohrmann, 1990) were observed 42 and 120 nt upstream of the translational start codon, respectively (Fig. 2c). No TATA box sequences could be identified. Immediately after the stop codon the nucleotide sequence was relatively AT rich. A consensus poly(A) signal (AATAAA) was first seen 639 nt downstream of the translational stop codon (data not shown). The presumed transcribed sequence TTAT was located 79 nt downstream of the translational stop codon in the SpliMNPV genome (Croizier et al., 1989) and cloned and sequenced (Fig. 1b). The SpliMNPV rr1orf was localized on the HincIII fragment of the SpliMNPV genome (Croizier et al., 1989) and sequenced (data not shown). The presumed translational start codon was in agreement with the Kozak consensus rule (Kozak, 1983). The putative start codons of the SpliMNPV rr1 gene and polyhedrin gene were separated by an intergenic region of 516 nt. The SpliMNPV rr1orf stop codon and a putative downstream ORF (data not shown) were separated by 117 nt.

An rr1 gene was also identified in SpliMNPV by hybridization with an SeMNPV rr1 DNA probe (data not shown). The SpliMNPV rr1orf was localized on the HindIII-E fragment of the SpliMNPV genome (Croizier et al., 1989) and cloned and sequenced (Fig. 1b). The SpliMNPV rr1orf consisted of 2345 nt with the potential to encode a protein of 781 amino acids with a predicted molecular mass of 87 kDa. Upstream of the presumed translational start site no consensus late baculoviral transcription start site was observed 79 nt in front of the translational start codon. Following the translation stop codon, three consensus poly(A) signals (AATAAA) were found. The ATG of the SpliMNPV rr1orf was in a favourable translational context (Kozak, 1983). The SpliMNPV rr1orf preceded an ORF with homology to the AcMNPV p74 gene (Kuzio et al., 1989). The SpliMNPV rr1orf stop codon and the presumed SpliMNPV p74 start codon were separated by 101 nt.

### Transcriptional activity of the SeMNPV rr1 gene

To investigate transcription of the SeMNPV rr1gene, a Northern blot with total RNA isolated from SeMNPV-infected cells at several times p.i. was hybridized to an rr1-specific probe (Fig. 2a). The SeMNPV rr1gene transcript was approximately 2.7 kb in size and present throughout infection, as observed after overexposure of the autoradiogram (data not shown), thus classifying rr1 as an early gene. The amount of transcript increased at later time-points.

The transcriptional start of the SeMNPV rr1gene was determined by primer extension (Fig. 2b). At early (4 h) and late (12, 24 h) times p.i., several transcriptional start sites were used. The major early start site was located at the A of the sequence TTATTT at nt −163 with respect to the translational start codon. At later times p.i. this site was also used. In addition, major late transcriptional start sites were observed at GACGAC (nt −129), at GGCATAA (nt −107) and at TTATTT (nt −78 and −75). The consensus late promoter sequence AAATAG (nt −120) was also used late in infection, although much less frequently than the other late start sites. Overexposure of the primer extension autoradiogram showed no indication of the use of this site at 4 h p.i., which also showed that the observed bands in the primer extension analysis were not due to preliminary stops. Comparable results were obtained with primer extension experiments at different annealing temperatures (data not shown). The length of the SeMNPV rr1transcript (2.7 kb) was in reasonable agreement with the length of the ORF (2.3 kb), assuming a poly(A) tail of normal length (0.2 kb), a 5′ leader of 74–163 nt and transcription termination near the translational stop codon.

### Comparison of baculoviral RR1 proteins with those from other sources

The amino acid sequences of SeMNPV and SpliMNPV RR1 were compared with those from other organisms and viruses. The overall homology (identity and similarity) is given in Table 1. The baculovirus RR1s have the highest homology to RR1s from eukaryotic organisms. The homology of SpliMNPV RR1 to these RR1s is higher than that of SeMNPV RR1. The homology between SeMNPV and SpliMNPV RR1, 69%, is essentially the same as that between SeMNPV and eukaryotic RR1s. The homology with RR1s from other DNA viruses is usually lower.

### Table 1. Percentage identity and similarity (in bold) of SeMNPV and SpliMNPV RR1 with RR1 proteins from several organisms and viruses

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See Fig. 3 legend for abbreviations.

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Fig. 3. For legend see p. 2372.
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SeMNPV RR1 had diverged in several regions of the protein and showed a few gaps and amino acid insertions when compared with eukaryotic RR1s. Several consensus amino acid residues (Mao et al., 1992; Stubbe et al., 1990; Uhlin & Eklund, 1994) could be recognized in the baculoviral RR1. These included cysteine residues...
Fig. 3. Alignment of the predicted amino acid sequences of the RR1 proteins of the baculoviruses SeMNPV and SpliMNPV with those of Homo sapiens (Hs; Parker et al., 1991), Caenorhabditis elegans (Ce; Sulston et al., 1992), Plasmodium falciparum (Pf; Rubin et al., 1993), Schizosaccharomyces pombe (Sp; Fernandez-Sarabia et al., 1993), vaccinia virus (VV; Tengelsen et al., 1988), African swine fever virus (ASFV; Boursnell et al., 1991), herpes simplex virus type 1 (HSV1; Nikas et al., 1986), herpesvirus saimiri (HVS; Nicholas et al., 1992), bacteriophage T4 (Tseng et al., 1988) and E. coli (Ec; Nilsson et al., 1988). Gaps introduced to optimize alignment are indicated by dots. Shading is used to indicate the occurrence (black, 100%; grey, at least 60%) of identical amino acids. Asterisks indicate essential cysteine residues. Alpha helices involved in dimerization are underlined (see text).

Fig. 4. Alignment of predicted amino acid sequences of the N terminus of the RR1 proteins of SeMNPV, SpliMNPV, Homo sapiens and Caenorhabditis elegans with those of the baculoviruses MbMNPV, PMNPV and OpSNPV. Shading indicates the occurrence of identical amino acids.
known to be involved in the formation of essential thiols in the *E. coli* RR1 protein, i.e. Cys-225, Cys-439, Cys-754 and Cys-759 (*E. coli* numbering), as well as residues surrounding Cys-225, Cys-439 and Cys-462 in the active site and two adjacent tyrosine residues (Tyr-730 and Tyr-731) presumably involved in electron transfer from the small subunit.

Structural analysis of the *E. coli* RR1 protein (Uhlin & Eklund, 1994) and previous research (Davis et al., 1994; Mao et al., 1992) revealed areas responsible for dimerization of RR1 and holoenzyme formation with RR2 subunits. The dimerization domains (two alpha helices, αA and βB) were well-conserved in SpliMNPV RR1 and slightly less conserved in SeMNPV RR1. Holoenzyme formation involves binding of the ultimate C-terminal residues of RR2 to two RR1 alpha helices (α1 andα13). The baculoviral RR1s showed reasonable sequence homology to the corresponding eukaryotic regions.

Databank searches revealed the occurrence of RR1-like sequences upstream of the polyhedrin gene in *Mamestra brassicae* multicapsid NPV (MbMNPV; Cameron & Possee, 1989), *Panolis flammea* multicapsid NPV (PfMNPV; Oakey et al., 1989) and *Orgyia pseudotsugata* single nucleocapsid NPV (OpSNPV; Leisy et al., 1986). The predicted amino acid sequences in these viruses showed homology to the N terminus of RR1 (Fig. 4). MbMNPV and PfMNPV RR1 showed the same gap around position 110 in amino acid alignment as SeMNPV RR1 in comparison with eukaryotic RR1. However, the available sequences from MbMNPV and PfMNPV showed a somewhat higher homology to the eukaryote RR1 N terminus than did SeMNPV RR1.

**Phylogenetic reconstruction of the origin of the baculoviral rr1 genes**

In order to investigate the origin of the *rr1* gene in SeMNPV and SpliMNPV, a phylogenetic tree was constructed with amino acid sequences of 30 RR1 proteins from prokaryotes, eukaryotes and viruses. An unrooted parsimonious tree was calculated with the PAUP heuristic search algorithm, followed by bootstrap analysis to assess the variability of the produced phylogeny (Fig. 5).

The tree showed that prokaryotes and eukaryotes, as expected, were located on separate branches. Viral RR1 sequences were found in many different locations in the tree. The herpesvirus RR1s were all clustered, implying that they diverged from a common ancestor. Bootstrap analysis supported the phylogenetic separation of the herpesviruses into α- and γ-herpesviruses.

Both SeMPNV and SpliMNPV grouped with eukaryotic organisms. The phylogeny of this branch was reasonably well-supported by bootstrap analysis. Tree data suggest that SeMNPV and SpliMNPV RR1 do not share a recent common

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(Rubin et al., 1993); ASFV, African swine fever virus (Boursnell et al., 1991).

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**Fig. 5.** Bootstrap analysis (100 replicates) of an unrooted phylogenetic tree of RR1 proteins constructed with PAUP heuristic search algorithm. Numbers at the branches indicate branch length (normal print) and frequency of cluster (italics). MtIB, *Mycobacterium tuberculosis* rrDE (Yang et al., 1994); StIB, *Salmonella typhimurium* rrDE (Jordan et al., 1994); Mg, *Mycoplasma genitalium* (Fraser et al., 1995); Bs, *Bacillus subtilis* (accession no. Z68500); Ec, *Escherichia coli* (Fleischmann et al., 1995); T4, *bacteriophage T4* (Tseng et al., 1988); HSV1, herpes simplex virus type 1 (Nikas et al., 1986); HSV2, herpes simplex virus type 2 (Swain & Galloway, 1986); EHV1, equine herpesvirus type 1 (Telford et al., 1992); EHV4, equine herpesvirus type 4 (Riggio & Onions, 1994); PRV, pseudorabies virus (Kaliman et al., 1994); BHV1, bovine herpesvirus type 1 (Simard et al., 1995); VZV, varicella zoster virus (Davison & Scott, 1986); GHV, gallid herpesvirus 2 (Darteil et al., 1995); HVS, herpesvirus saimiri (Nicholas et al., 1992); EHV2, equine herpesvirus type 2 (accession no. U20824); EBV, Epstein-Barr virus (Baer et al., 1984); Sc, *Saccharomyces cerevisiae* (Elledge & Davis, 1990); Sdln, *Saccharomyces cerevisiae* DNA damage inducible subunit (Yagle & McIntee, 1990); Sp, *Schizosaccharomyces pombe* (Fernandez et al., 1993); VV, vaccinia virus (Tengelsen et al., 1988); Var, variola virus (Shchelkunov et al., 1993); Hs, *Homo sapiens* (Parker et al., 1991); Mm, *Mus musculus* (Caras et al., 1985); Ce, *Caenorhabditis elegans* (Sulston et al., 1992); Pf, *Plasmodium falciparum*.
ancestor, but that these viruses acquired the gene for RR1 independently from each other as well as from other DNA viruses.

Discussion

The complete nucleotide sequence of the rr1 gene was determined in the baculoviruses SeMNPV and SpliMNPV. Transcriptional activity was investigated after RNA isolation from SeMNPV-infected cultured insect cells. Both Northern blotting and primer extension showed low transcriptional activity early in infection, which increased at later stages. The early transcription of the SeMNPV rr1 gene is in good agreement with its presumed role in deoxyribonucleotide synthesis required for DNA replication. Herpesvirus and vaccinia virus rr genes are also transcribed as early genes (Schmitt & Stunnenberg, 1988; Swain & Galloway, 1986).

The sequence surrounding the major transcriptional start sites showed no homology to previously characterized start sites of other baculovirus genes, for instance those involved in DNA replication, such as helicase (Lu & Carstens, 1992) and DNA polymerase (Tomasli et al., 1988). No homology between the sequences 5′ of the translational start of SeMNPV, SpliMNPV and other baculovirus rr1 genes could be detected.

The alignment of SeMNPV and SpliMNPV RR1 with other RR1s (Fig. 3) showed that amino acid residues known to be involved in enzymatic activity were conserved. This suggests that the baculoviral rr1 gene could code for a functional constituent of an RR enzyme. SeMNPV RR1 had a lower homology to eukaryotic RR1 than SpliMNPV RR1. In comparison to the E. coli structural regions (Uhlin & Eklund, 1994), regions from eukaryotic and specifically viral RR1 which diverged from the E. coli sequence, often map to loops separating α-helices and β-barrels. One of the gaps in the SeMNPV RR1 alignment was located around amino acid 250. A similar situation was observed in several other viral RR1s. In E. coli, this region forms a loop (L1, amino acids 259–277) between the dimerizing domains of RR1. It has been suggested that this and other loops in the dimerization region may change from a flexible to a fixed position at subunit formation, hence stabilizing the holoenzyme (Uhlin & Eklund, 1994). The SeMNPV enzyme may thus show a stability different from that of the cellular enzyme.

Sequences with homology to the RR1 N terminus were found in the baculoviruses MbMNPV, PIMNPV and OpSNPV (Fig. 4). This suggests that RR1 is not only encoded by SeMNPV and SpliMNPV, but is widespread among baculoviruses. The rr1 gene in MbMNPV, PIMNPV and OpSNPV baculoviruses had a similar genomic location to that from SeMNPV, i.e. positioned upstream of the polyhedrin gene. Phylogenetic trees based on polyhedrin gene sequences (Zanotto et al., 1993; Cowan et al., 1994) placed SeMNPV, MbMNPV and PIMNPV in subgroup IIA. The position of OpSNPV was variant depending on which algorithms and DNA or amino acid sequences were used. OpSNPV was either in group IIB, together with SpliMNPV, or in IIA. OpSNPV might belong to subgroup IIA, based on the observed homology to the RR1 N terminus and its genomic location next to the polyhedrin gene.

Bootstrap analysis of the parsimonious phylogenetic tree (Fig. 5) confirmed the independent ancestry of the rr1 gene in SeMNPV and SpliMNPV. This conclusion is in agreement with the different genomic location of the rr1 gene in these two viruses, as well as with the observed lower homology of SeMNPV RR1 with eukaryotic RR1s. Alternatively, if the common ancestor of SeMNPV and SpliMNPV already encoded RR1, the implication would be that the rr1 gene evolved much faster in SeMNPV than in SpliMNPV. This assumption seems questionable and is not supported by determination of the evolutionary rates of the polyhedrin genes of SeMNPV and SpliMNPV (Zanotto et al., 1993; Cowan et al., 1994), nor by comparison of other genes of SeMNPV and SpliMNPV (data not shown). However, the phylogenetic reconstruction may yield a different result when full-length sequences of other baculovirus rr1 genes become available.

The positioning of SeMNPV and SpliMNPV in the eukaryote branch of the tree suggests that each virus derived RR1 independently from an eukaryotic source, for example their host. The polyphyletic origin of baculovirus RR1 contrasted with the observed monophyletic grouping of the herpesviruses and of the two poxviruses. Only RR1 from SpliMNPV and poxviruses grouped inside a cellular clade, whereas RR1 from SeMNPV, ASFV, herpesviruses and bacteriophage T4 diverged more than their presumed ancestors did. This suggests that viral RR1 usually diverges faster than cellular RR1.

RR1 proteins need to associate with RR2 dimers in order to form a functional enzyme. In most large DNA viruses (with the exception of vaccinia virus) the genes for RR1 and RR2 are located adjacent to each other. A different situation exists in β-herpesviruses such as human cytomegalovirus, which only contain a RR1-like ORF (Chee et al., 1990). However, unlike the baculoviral RR1 ORFs, the RR1-like ORFs in β-herpesviruses show only very limited homology to other RR1s and do not encode the amino acid residues known to be involved in ribonucleotide reduction. It has been suggested that these highly diverged genes may have acquired another function during the β-herpesvirus life cycle (Conner et al., 1994). In SeMNPV, homology to RR2 could not be detected either in the 2.5 kb sequence upstream or in the 14 kb sequence downstream of the SeMNPV RR1 ORF (data not shown). However, RR2 might be located elsewhere on the genome. As yet, we do not know if SeMNPV and SpliMNPV encode their own RR2, or alternatively, combine with cellular RR2 proteins. Such a heterologous association is known in yeast, where two RR enzymes are recognized. One enzyme results from the combination of RR1 and RR2, whose normal expression is
restricted to certain phases of the cell cycle. The other enzyme is induced by DNA damage and is a combination of the same RR2 and a different RR1 (Elledge et al., 1992).

Virus-encoded RR enzymes from poxviruses, herpesviruses, ASV and phage T4 are not essential and are distinct from the cellular enzyme in several aspects. It remains to be elucidated if similar characteristics hold for baculoviruses encoding or lacking RR.

Not all large DNA viruses encode RR. As can be concluded from sequence analysis of the complete genome, the baculovirus type species AcMNPV does not encode RR (Ayres et al., 1994) and neither does the closely related Bombyx mori NPV (accession number L33180). This explains our failure in attempts to hybridize an SeMNPV rr1 DNA probe to AcMNPV DNA (data not shown). The herpesvirus channel catfish virus (Davison, 1992) also does not encode RR. The reasons for this are unknown. The lack of rr1 in AcMNPV will allow the study of SeMNPV rr1 in an AcMNPV recombinant expressing this gene.

During the course of our experiments, Ahrens et al. (1997) published the entire sequence of the Orgyia pseudotsugata multicapsid nucleopolyhedrosis virus (OpMNPV) genome and also identified an rr1 gene, which potentially encoded a relatively short protein of 593 amino acids. In juxtaposition with the OpMNPV rr1 gene, a gene with low sequence homology to RR2 was found in this baculovirus. Preliminary phylogenetic analysis indicated that the OpMNPV rr1 gene did not share a recent common ancestor with either SeMNPV or SpliMNPV, but was acquired independently in these three baculoviruses.

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References


Baculovirus ribonucleotide reductase large subunit


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