The Molecular Biology of Baculoviruses

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Preface

The pathology caused by baculoviruses in insect populations was described centuries ago, notably in the larvae of insects such as the silkworm (*Bombyx mori*) which has been appreciated for the quality and beauty of its products. In the 1940s baculoviruses and their structure and physiology were intensively investigated, particularly by Bergold's group in Tübingen. The following decades saw excellent progress, laying a solid virological base for later investigations on the system. Further studies mushroomed in the 1970s with the advent of tissue culture systems for insect cells which eventually facilitated the molecular biological approach that came to the fore in the 1980s.

One of the reasons for pursuing research on the baculovirus system was the prospect of eventually using these viruses as insect pest control agents. While this practical aspect may appeal to many, molecular biologists had additional reasons to be interested in baculoviruses. Here was a large DNA viral genome, probably fraught with problems of replication and regulation that hopefully would open inroads into the molecular biology of interesting insect cell systems. In the days when genetechnology promises laurels, and after several virus systems had been skilfully exploited as highly efficient eukaryotic expression vectors, it came as no surprise that baculoviruses were also investigated in that respect. Indeed, the *Autographa californica* nuclear polyhedrosis virus became a good vector. Insect cells also seem to collaborate in modifying and processing the genetechnologically synthesized polypeptides. History went a full cycle, as it were, when it was demonstrated how man's faithful working horse, the silkworm, could now be exploited to reap the fruits of baculovirus genetechnology.

The objective of this volume is not to present an encyclopedia of baculoviruses, but rather to offer a compendium for the interested student of baculovirology with current contributions from laboratories which are actively involved in work on the molecular biology of these viruses. The following topics will be considered:

Structure and replication of baculoviruses, the structure of the viral genome and its expression as well as the regulation of baculovirus gene expression are the topics of several chapters. One of these chapters also summarizes the current information on foreign genes that have been expressed by the baculovirus vector system. Another chapter is devoted to the molecular biology of granulosis viruses. It has been recognized for some time that insect cells can be persistently infected with baculoviruses, and the present state of investigations on this topic is the subject of another chapter. Detailed analyses on virus-specific proteins, like the 64-kilodalton envelope protein and the inclusion body protein, the polyhedrin, will also be presented. Biochemical analyses on the structure and function of virus-encoded proteins will certainly constitute a very important area for further research.

It is hoped that the papers collected in this volume will be useful for newcomers to the field seeking to familiarize themselves with current research work, as well as for specialists seeking detailed information. These reviews do not intend to treat baculovirology comprehensively. There will be other sources of information to fulfill that request. I am aware of the shortcoming that certain important areas of molecular biology, e.g., the problem of baculovirus DNA replication, have hardly been touched upon. Frankly, there is not enough definitive information from recent work to warrant a full chapter. Future books on the molecular biology of baculoviruses will hopefully help to alleviate some of these unavoidable omissions.

We would like to thank the authors who have contributed to this volume and transgressed the deadline only minimally or not at all. It is a pleasure to thank Springer-Verlag, in particular Marga Botsch, for their help and excellent collaboration.

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An Overview of the Structure and Replication of Baculoviruses

P. FAULKNER and E.B. CARSTENS

1 Introduction

Diseases of insects, in particular those of the honeybee and the silkworm, have been recognized since ancient times and were the subject of speculation in historical writings. According to BERGOLD (1953), MAESTRI and CORNELIA in 1856 established a correlation between the presence of crystalline occlusion bodies within tissues of insects and the symptoms of disease in silkworms. A similar pattern of disease was seen in forest insects in the early part of this century, and the infections became known as nuclear polyhedrosis diseases. General acceptance that the agent was viral in nature followed demonstrations that extracts of silkworm tissue from jaundiced larvae retained infectivity after passing through an ultrafilter (VON PROWASEK 1912; ACQUA 1918). In 1947 BERGOLD demonstrated that rod-shaped virions could be released from occulusion bodies in the presence of dilute alkali (BERGOLD 1947) and that they were enveloped and contained DNA. Since that time there has been a continuing scientific interest in furthering knowledge of the part these viruses play in natural control of insect populations. Detailed biochemical studies on the nature of the virus

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and its molecular biology have accumulated in recent years. Much of the current thrust of research is directed to assessing the role of the viruses in integrated insect pest management programs and, at the molecular level, in attempts to engineer viruses of altered insect host range and virulence. Other research is directed to utilizing baculoviruses as expression vectors for production of non-baculovirus proteins (MILLER et al. 1982; 1983a).

The family Baculoviridae contains a single genus, *Baculovirus*, which is divided into three subgroups (MATHEWS 1982). (1) Subgroup A contains the nuclear polyhedrosis viruses (NPV). Nuclei of infected cells become filled with crystalline polyhedron-shaped occlusion bodies (OB) which are up to 15 μm in size and are easily seen in the light microscope. About 95% of the mass of the OB is made up of a crystalline protein lattice in which are embedded enveloped virus nucleocapsids. The viruses are dispersed throughout the OB either as separate enveloped nucleocapsids (SNPV) or as bundles of one to seven nucleocapsids within a common envelope (MNPV). (2) Subgroup B consists of the granulosis viruses (GV); here the OB are much smaller (about 0.5 μm) and are ellipsoidal in shape. They occlude a single enveloped virus particle. (3) The type virus in subgroup C is *Oryctes* baculovirus which infects the rhinoceros beetle. The virion is a rod-shaped, enveloped nucleocapsid which does not become occluded and is transmitted as the free particle.

Most investigations on gene expression of baculoviruses have been done using *Autographa californica* nuclear polyhedrosis virus (AcMNPV). Studies have also been carried out on other baculoviruses, particularly those which infect pest insects and result in agricultural crop or forest damage. Recent reviews on baculovirus structure and genome organization and expression are by HARRAP and PAYNE (1979), MILLER (1984), KELLY (1985), and COCHRAN et al. (1986).

2 The Infectious Cycle

Permissive cell lines (reviewed in KNUDSON and BUCKLEY 1977 and PRISTON 1985) have been used for over a decade to study the morphogenesis and biochemistry of replication of nuclear polyhedrosis viruses. Recently cell cultures have been developed which support a codling moth granulosis virus (NASER et al. 1984; MILTENBERGER et al. 1984) and two subgroup C nonoccluded baculoviruses: from the rhinoceros beetle (CRAWFORD and SHEEHAN 1985) and from *Heliothis zea* (RALSTON et al. 1981).

The replication of NPV in cell culture is biphasic and two phenotypes of the virion are generated (VOLKMAN et al. 1976) in the infectious cycle (reviewed in: GRANADOS 1980; FAULKNER 1981; VAUGHN and DOUGHERTY 1985). AcMNPV infection of cell cultures is initiated by the nonoccluded form of the virus (NOV). The virion, which is an enveloped nucleocapsid, becomes internalized by the process of adsorptive endocytosis (VOLKMAN and GOLDSMITH 1985). Uncoated nucleocapsids traverse the cytoplasm and either enter the nucleus or discharge the genome at nuclear pores. Virus entry appears to be an

efficient process since nucleocapsids have been seen in the nucleus within 1 h of infection (BASSEMIR et al. 1983). Transcription and DNA replication occur within the nucleus and unenveloped nucleocapsids are observed within the first 12 h postinfection. Morphogenesis of the nucleocapsid apparently first involves assembly of capsids followed by addition of nucleoprotein to the capsid shells (BASSEMIR et al. 1983). In virus morphogenesis the nucleocapsids are first observed lying within patches of DNA-rich virogenic stroma which form in infected nuclei. Early in infection, newly formed unenveloped nucleocapsids traverse the cytoplasm and progeny NOV are released by budding at the plasma membrane (GRANADOS 1980; FAULKNER 1981). In the second phase of the infectious cycle budding of NOV appears to be reduced (VOLKMAN et al. 1976) and *de novo* membrane proliferation may occur within the nucleus (STOLTZ et al. 1973). Bundles of enveloped nucleocapsids accumulate in the nucleus and then become encapsulated within the matrix of the occlusion bodies. Occlusion of the newly assembled occluded virus (OV) is by crystallization of polyhedrin around the bundles (CHUNG et al. 1980).

In nature the two baculovirus phenotypes have different functions in the replication and transmission of the virus. Larvae of Lepidopteran insects become infected when baculovirus occlusion bodies are ingested along with the food. They dissolve at about pH 10.5 in gut juice and bundles of OV are released. The OV infect gut epithelial cells and virus replication takes place. Enveloped nucleocapsids of NOV phenotype are budded from the basal surface of gut cells into the hemocoel (ADAMS et al. 1977). A generalized dispersion of the virus follows, with many tissues becoming infected. Secondary rounds of replication occur and ultimately occlusion bodies form within nuclei in most tissues of the larvae (FAULKNER 1981).

3 Properties of the NOV and OV Phenotypes

Envelopes and capsids of NOV and OV differ in polypeptide (VOLKMAN 1983) and glycoprotein (STILES and WOOD 1983) composition but the genomes from both phenotypes have a similar DNA sequence (COCHRAN et al. 1982). A recent study of surface envelope proteins by lactoperoxidase iodination identified at least 12 polypeptides in AcMNPV NOV and 13 in the OV form. Eight of the NOV but none of the OV polypeptides were glycosylated (STILES and WOOD 1983). The phenotypes have differing infectivities according to the route of administration. Under experimental conditions OV have high infectivity *per os* but are of low infectivity if injected into the hemocoele. In contrast NOV are of low infectivity per os but highly infectious when injected (DOUGHERTY et al. 1975; KEDDIE and VOLKMAN 1985).

Envelopes of both phenotypes of baculoviruses can be removed by detergent treatment. The nucleocapsids are rod shaped and about 40 nm in diameter and 300 nm long. Further treatment of the nucleocapsids with dilute alkali or with detergent and high salt (WILSON and CONSIGLI 1985b) separates the capsids and virus cores. Capsids retain the cylindrical form of the nucleocapsid,

and a lattice pattern of capsomers is seen in the EM (HARRAP 1972; BEATON and FILSHIE 1976). The predominant capsid polypeptide is 44K in size (SUMMERS and SMITH 1978), but up to eight minor polypeptides may also be present (KELLY 1985). Purified capsids of a GV virus have protein kinase activity (WILSON and CONSIGLI 1985b). The enzyme requires Mn^{2+} for activation and can phosphorylate the 13K virus core DNA-binding protein. Similar basic proteins have been isolated from NPV and other GV baculoviruses (TWEETEN et al. 1981; KELLY et al. 1982). It has been postulated that during cellular infection the 13K protein is phosphorylated by a capsid protein kinase and that virus DNA is released from the core complex. WILSON and CONSIGLI (1985) have proposed that in GV infections nucleocapsids become aligned with nuclear pores and protein kinase is activated. The action of the enzyme would then be to allow for release of genomic DNA into the nucleus to initiate infection.

4 The Baculovirus Genome

The genome of the baculoviruses is a closed circular superhelical molecule of double-stranded DNA. Genomes ranging in size from 60 to 250 kbp have been reported (MILLER 1984), but the median is about 100 kbp. The general conformation was established based on biophysical properties, e.g., behavior of virion DNA in density gradients in the presence of intercalating agents and by direct observation in the electron microscope of DNA released from virus particles. (Some recent reviews are: HARRAP and PAYNE 1979; FAULKNER 1981; MILLER 1984; KELLY 1985.) The degree of supercoiling has not been accurately determined because most DNA extracted from virions is in the form of nicked (relaxed) circles. The virion vDNA is believed to be in tight association with a strongly basic polypeptide of approximately 13K molecular weight which stabilizes the DNA within the nucleocapsid (TWEETEN et al. 1981b; KELLY et al. 1982).

4.1 The Physical Map

Development of a physical map provided an important step in elucidating the gene order in baculoviruses. In a physical map, DNA fragments generated with class II restriction endonucleases (REN) are ordered on a linear or circular scale. By convention the scale is usually divided into 100 units and the actual location of REN fragments or specific sequences are reported as a range within the scale of 0–100 map units. Strategies used in developing physical maps of baculoviruses have been reviewed by MILLER (1984) and by COCHRAN et al. (1986).

The first physical maps of the baculovirus genome were with variants of AcMNPV (E-2, SMITH and SUMMERS 1979, VLAK 1980; L-1, MILLER and DAWES 1979; HR-3, COCHRAN et al. 1982; and D, LUBBERT et al. 1981). The circular nature of the genome was confirmed in these studies and estimates of the molecu-

lar weight of the genome were obtained based on summation of the sizes of the REN fragments. The physical map was oriented and the 0/100 locus defined in keeping with a consensus proposal among baculovirologists (VLAK and SMITH 1982). The map originated and ended at the *Eco*R1 B/I junction of the E-2, L-1, and HR-3 variants and the B/H junction of the D variant. The rationale for using the *Eco*R1 B/I junction for orientation was based on studies which located the polyhedrin gene within the *Eco*R1-I fragment of the E-2 variant (SUMMERS et al. 1980). The AcMNPV map has been utilized in studies on gene composition and expression. Portions of it have been finemapped and sequenced (HOOFT VAN IDDEKINGE et al. 1983; KUZIO et al. 1984).

In the past few years physical maps have been published for other baculoviruses and their strain variants. The list includes the AcMNPV variants: *Spodoptera exempta* MNPV (BROWN et al. 1985), *Galleria melonella* MNPV, and *Trichoplusia ni* MNPV (SMITH and SUMMERS 1979). In addition physical maps have been published on MNPV isolated from *Choristoneura fumiferana*, CfMNPV (spruce budworm) (ARIF et al. 1984), *Orgyia pseudotsugata*, OpMNPV (tussock moth) (LEISY et al. 1984), *Spodoptera frugiperda*, SfMNPV (MARUNIAK et al. 1984), and *Mamestra brassicae* (WIEGERS and VLAK 1984). Among SNPV, genomes of baculoviruses from *Heliothis zea* HzSNPV (KNELL and SUMMERS 1984), assorted Plusiine hosts (BILIMORIA 1983), and *Orgyia pseudotsugata* have been subjected to REN analysis. GVs from *Plodia interpunctella* and *Pieris rapae* (TWEETEN et al. 1980a) and the genome of the nonoccluded group C *Oryctes* baculovirus (CRAWFORD et al. 1985) have also been analyzed.

4.2 Variants and Strains Related to AcMNPV

As attention focussed on details of the physical map of AcMNPV it became apparent that cloned isolates of the virus often differed from the prototype strain in the fine structure of the genome. The differences in DNA sequence may be reduplication of portions of the genome, deletions of sequences, and base substitutions which may change the REN pattern of the isolate. Variations of genome structure have been observed in field isolates of AcMNPV (LEE and MILLER 1978; SMITH and SUMMERS 1979; TJIA et al. 1979; KNELL and SUMMERS 1981), *Cydia pomella* GV (HARVEY and VOLKMAN 1983), and other baculoviruses. They have also been induced in cell culture, especially following serial passage of AcMNPV (FRASER and HINK 1982; FRASER et al. 1985) or by subjecting the replicating virus to mutagens during replication (ERLANDSON et al. 1984). Instances of insertion of host cell DNA including the insertion of transposon-like elements have also been reported when the virus is grown in cell culture (MILLER and MILLER 1982; FRASER et al. 1983). A comprehensive physical map describing the variations found in numerous isolates of AcMNPV is given in the review by MILLER (1984).

Observations on variations in the physical map of AcMNPV have raised questions on the meaning of species, strain, and variant in the taxonomy of the baculoviruses. Until recently it has been the practice to name baculoviruses after the insect from which they were originally isolated (TINSLEY and HARRAP

1978); thus, NPVs derived from *Autographa californica, Galleria melonella, Rachoplusia ou,* and *Trichoplusia ni* were treated as separate species. It has been shown that these viruses differ only slightly in biological properties and their genomes have only minor variations in their physical maps (SMITH and SUMMERS 1979; SUMMERS et al. 1980). The use of Southern blotting and dot-blotting techniques has helped delineate the close similarity between many AcMNPV variants (SMITH and SUMMERS 1982). Investigations of such biological properties as host range and virulence have also often borne out these close relationships (HARVEY and VOLKMAN 1983). The taxonomic problem of distinguishing betwen baculovirus strains and variants remains. A useful approach is taken by COCHRAN et al. (1986), who point out that variants may be considered to be isolates derived from the same insect host, while strains are isolates derived from other insect species. Thus the four AcMNPV variants, E-2, HR-3, L-1, and D, were all isolated and cloned from early passage stocks of field collections of *Autographa californica.* However, there are only minor differences in the physical maps and REN profiles of AcMNPV, TnMNPV, and Se-25MNPV (BROWN et al. 1984), but these would be considered as strains since they were isolated from different insect species. In practice investigators need only be aware that divergences from a "prototype" sequence are often observed in a working strain. Nowadays most molecular genetic studies involve fine mapping of a locus within the genome and precise definitions of gene structure are given in each investigation.

4.3 Reiterated DNA Sequences Within Genomes

The genome of AcMNPV possesses five regions (HR 1–5) which show DNA homology when examined by Southern blotting under stringent hybridization conditions. The homologous regions are 350–500 bp in size and are rich in *Eco*R1 sites (COCHRAN and FAULKNER 1983). Altogether they represent about 3% of the total genome size. The homologous regions were detected by Southern blot and cross-blot hybridization and regions of homology were within the *Hind*III-A, -K, -L, -Q fragments. The *Hind*-L, -Q fragments were digested with *Eco*R1 and cloned into the plasmid pBR322. The cloned "minifragments" were shown to hybridize to all five homologous regions of the AcMNPV genome.

Regions of DNA homology have also been found in *Choristoneura fumiferana* NPV (ARIF and DOERFLER 1984) and possibly in *Orgyia pseudotsugata* NPV (LEISY et al. 1984). In addition, the subgroup-C *Oryctes* baculovirus contains four regions of sequence homology (CRAWFORD et al. 1985).

Intragenic reiterated sequences from a given baculovirus genome do not seem to be conserved in DNAs of other baculovirus species. Thus, although extensive homology has been noted between the genomes of AcMNPV and CfMNPV (ARIF and BROWN 1975) the homologous regions from the two viruses do not share the same sets of reiterated sequences (COCHRAN et al. 1986). The biological significance of the homologous repeat regions is largely unknown. The HR-5 region of AcMNPV is not essential for replication of the virus in the cell culture since a deletion mutant lacking it grew normally (J. KUZIO,

personal communication). HR-5 is flanked by a transcriptionally active locus in the AcMNPV genome (LUBBERT and DOERFLER 1984b; FRIESEN and MILLER 1985; RANKIN et al. 1986) and may possibly have a role to play in regulating adjacent genes.

In addition to the homologous regions the genomes of baculoviruses may also generate insertions, reduplications of sequences, and transposon-like elements with flanking LTRs following continuous passage in cell culture (MILLER and MILLER 1982; FRASER and HINK 1982; FRASER et al. 1983). Should insertional mutagenesis of transposons occur, major effects on gene regulation within the virus could be anticipated. There are no reports of acquisition of host DNA sequences in wild-type isolates of baculoviruses.

4.4 Conservation of DNA Sequences Between Genomes

There have been several reports of intergenic homology among baculoviruses. Homologies were detected among Lepidopteran NPVs (JEWELL and MILLER 1980) and, using dot-blot hybridization at low and high stringenices, between a large group of MNPVs, SNPVs, GVs and a subgroup-C baculovirus (SMITH and SUMMERS 1982). The polyhedrin gene and a region encompassing the *p10* gene are highly conserved among NPVs infecting Lepidoptera.

Recent findings of homologies between large segments of the genomes of AcMNPV and OpMNPV (LEISY et al. 1984), AcMNPV and CfMNPV (ARIF et al. 1985), and AcMNPV and HzSNPV (KNELL and SUMMERS 1984) have indicated that the three viruses are colinear with respect to their gene distribution. The data point to strong evolutionary pressure that has preserved the structural organization of baculovirus genomes. Other evidence for conservation of DNA structure among the baculoviruses comes from studies made by Rohrmann and colleagues on the evolutionary history of the gene coding for the major occlusion body polyhedrin. Predictions of base sequence derived from N-terminal analyses of polyhedrins from baculoviruses derived from Lepidoptera, Hymenoptera, and Diptera, indicated major conservation of the gene through the evolutionary history of the viruses (ROHRMANN et al. 1981).

5 Replication of Baculovirus DNA

Baculoviruses have the largest circular virus genomes known. The genomes of several baculoviruses have been examined extensively and features which regulate transcription (ROHEL and FAULKNER 1984; ERLANDSON and CARSTENS 1983; ERLANDSON et al. 1985) and translation (ESCHE et al. 1982; VLAK et al. 1981) have been mapped in a few instances. However, little is known at present about the biochemical mechanisms by which the genome is replicated. Since purified viral DNA is infectious (CARSTENS et al. 1980; POTTER and MILLER 1980; BURAND et al. 1980), the capsid-free genome must be in a conformation ready for transcription as soon as it enters the cell nucleus. Whether this DNA associates with host cell proteins prior to transcription has yet to be determined

but it is likely that nucleosomes assemble to protect the DNA from nucleases prior to the initiation of newly synthesized virus DNA (L.K. MILLER, personal communication). At least one report has suggested that the input DNA can be reincorporated in progeny virions (DOBOS and COCHRAN 1980). In cell cultures vDNA synthesis begins about 6 h postinfection and proceeds for at least another 12 h (TJIA et al. 1979; ERLANDSON and CARSTENS 1983). There is probably a decrease in the rate of vDNA synthesis after 20 h postinfection (TJIA et al. 1979) although the kinetics have not been well studied. A specific endonuclease activity has been detected in infected cells. The enzyme degrades cellular but not virus DNA (KELLY and WANG 1981; BROWN et al. 1979). Its presence could explain the shut-off of transcription and translation of cellular genes observed at late times after infection (CARSTENS et al. 1979; VLAK et al. 1981; ESCHE et al. 1982; ROHEL et al. 1983; LUBBERT and DOERFLER 1984a). The identification of a virus gene product responsible for this inhibition of cellular processes has great potential for developing a strain of virus with enhanced virulence by increasing either the level of gene expression or the length of time that it is synthesized.

Prior synthesis of virus polypeptides is necessary for the initiation of vDNA replication (KELLY 1982; GORDON and CARSTENS 1984; ERLANDSON et al. 1985) although their identification and the role of these proteins in virus infection has not yet been determined. A partial inhibition of vDNA synthesis was seen when infected cells were treated with drugs such as cycloheximide, actinomycin D, and cytosine arabinoside which inhibit protein and DNA synthesis (KELLY and LESCOTT 1976). However, some vDNA synthesis continued even in the presence of high drug concentrations. A similar effect on the level of protein synthesis has also been observed (DOBOS and COCHRAN 1980). More sensitive techniques of analysis such as Southern and dot-blot hybridizations need to be utilized to clarify relationships between protein synthesis and vDNA replication. Such studies could, for example, investigate whether virus transcripts are produced in the absence of protein synthesis and whether inhibition of protein synthesis completely blocks DNA replication.

Another approach to study baculovirus DNA replication has utilized temperature-sensitive mutants which depress the synthesis of vDNA at the nonpermissive temperature. Two such AcMNPV mutants, ts8 and tsB821, have been described (BROWN et al. 1979; GORDON and CARSTENS 1984; MILLER et al. 1983b). Both grow normally at the permissive temperature but fail to synthesize significant levels of vDNA at the restricted temperature. Five virus-induced proteins have been detected in mutant-infected cells at the nonpermissive temperature. These appear to be early proteins which are normally produced prior to initiation of DNA replication. Both mutants have been mapped by marker rescue to different regions of the genome. The ts8 mutation maps within 60.1 and 62 map units (GORDON and CARSTENS 1984) and the tsB821 lesion maps between 90.7 and 1.9 map units (MILLER et al. 1983b). The 60.1–62 map unit region codes for a 4.6-kb transcript in Wt-infected cells (ERLANDSON et al. 1985) and may encode the protein defective in ts8. No specific RNA has been associated yet with tsB821 and no polypeptide has been assigned to the altered sites in either mutant. Recently, an immediate early gene product required for

the initiation of transcription from another other early promoter (for a p39 polypeptide) has been mapped between 95 and 97.5 map units (GUARINO and SUMMERS 1986). It remains to be determined whether this is the gene which is altered in tsB821.

Only one report has described a possible origin of replication on the genome of a baculovirus (BLINOV et al. 1984). Location of the tentative origin for GmMNPV (a variant of AcMNPV) was based on the ability of DNA from insect cells previously transfected with plasmids carrying vDNA fragments to transform *E. coli* competent cells. The authors found that the GmMNPV *Bam*HI fragment H carried an origin of replication. By comparing their fragment designations with published maps (SMITH and SUMMERS 1979), it is likely that the region of interest is homologous with the AcMNPV *Bam*HI G fragment. These results await confirmation. It would perhaps be coincidental that this region contains a legitimate baculovirus origin because other work suggests that virus-specific protein synthesis is required prior to the initiation of virus DNA synthesis in insect cells (KELLY 1982; ERLANDSON et al. 1985).

Another approach for the identification of potential baculovirus origins of DNA replication has utilized a yeast vector system to detect autonomous replicating sequences (ARS) in *Saccharomyces cerevisiae* (STRUHL et al. 1979). Baculovirus restriction fragments were cloned randomly into YIp5, and putative ARS were identified within the AcMNPV *Eco*RI K and *Eco*RI F fragments (HOOFT VAN IDDEKINGE et al., to be published). We have also identified yeast plasmid chimeras containing the AcMNPV *Eco*RI K fragment, which are capable of transforming *S. cerevisiae* (R. LO and E.B. CARSTENS, unpublished results). Thus far, there has been no direct demonstration that these ARS-containing regions carry natural origins of DNA replication used by the virus in permissive cells.

Aberrations in baculovirus DNA replication in cell culture have also been noted. It has been known for some time that phenotypic changes occur in the production of occlusion bodies following continued passage in cell culture (MACKINNON et al. 1974; POTTER et al. 1976; FRASER and HINK 1982). The change in phenotype from many polyhedra (MP) to few polyhedra (FP) per infected cell nuclei has, in some cases, been associated with genomic alterations (CROZIER and QUOIT 1981; MILLER and MILLER 1982; FRASER et al. 1983; CARSTENS et al. 1986). These have resulted from the insertion of host cell DNA sequences (MILLER and MILLER 1982; FRASER et al. 1983) into the viral genome or the insertion of repeated sequences of viral DNA (BURAND and SUMMERS 1982). A similar process may occur during replication of baculovirus in insects. In the laboratory the selection of FP variants is convenient since it is based on the observation of numbers of OB/per infected cell. FP variants are selected against in insects since the synthesis of fewer OB effectively lowers virulence. Isolates of baculovirus from insects consist of a mixture of genotypic variants (MILLER and DAWES 1978; SMITH and SUMMERS 1978; TJIA et al. 1979) and it is likely that other genomic variants occur during replication of baculovirus, for example those in which some vDNA sequences are reiterated. If these rearrangements result in a lower virulence for the virus, they would be selected against in an insect population.

Two other mutants have been described which demonstrate aberrations in DNA replication. Ts8 synthesizes DNA which contains copies of a 1.35-kbp sequence of viral DNA tandemly repeated in the 90-map-unit region of its genome (ERLANDSON et al. 1984). Variants which carry the insertions apparently are not viable since plaque purification does not result in the isolation of infectious virions missing the original non-repeated DNA sequence. The *Hind*III P fragment, including the 1.35-kb insert, has recently been sequenced (J. KUZIO, P. FAULKNER, C. CURRY, and E.B. CARSTENS, unpublished results) and an open reading frame potentially coding for a 74K protein has been identified. The reiteration in ts8 includes sequences completely within this open reading frame suggesting the presence of an essential gene in this location in the viral genome.

The morphology mutant M5 has also been shown to have an aberration in DNA replication. In this case, two size classes of viral DNA are synthesized, one of 100% and another of 58% of the normal genome length (CARSTENS 1982). The smaller DNA molecule results from the specific deletion (or lack of synthesis) of sequences between 2.6 and 47 map units. M5 carries two almost identical inserts of 290 bases of DNA at these two locations (CARSTENS 1982; E.B. CARSTENS, unpublished results). However, it is not known how the presence of these inserts affects the replication of the M5 genome.

6 Transcription in Baculoviruses

Most of published baculovirus transcription data is based on studies of AcMNPV expression. Biochemical analysis of AcMNPV mRNAs has revealed a cap structure on the 5′ termini, indicating a similarity to other eukaryotic mRNAs (JUN-CHUANG and WEAVER 1982). It is assumed that viral mRNAs are polyadenylated since both total cytoplasmic and poly (A)+ RNA give identical patterns in northern blot analyses (ERLANDSON et al. 1985) and give similar polypeptides in in vitro translation assays (VLAK et al. 1981). AcMNPV-infected cells express a unique RNA polymerase activity which is distinct from the host cell RNA polymerase II (GRULA et al. 1981). The presence of an alpha-amanitin-resistant RNA polymerase activity in infected cells suggests that the virus either codes for its own polymerase or it uses an enzyme other than the host RNA polymerase II for the majority of its transcription (VLAK et al. 1981). It appears that initially virus transcription must utilize host polymerases since purified virus DNA can initiate infections (CARSTENS et al. 1980; BURAND et al. 1980). Later in infection it is possible that virus-specific proteins modify the host polymerase, enabling virus promoters to be recognized (GUARINO and SUMMERS 1986). All the late baculovirus promoters that have been sequenced reveal similar upstream regulatory sequences such as TATA boxes and CAAT sequences (HOOFT VAN IDDEKINGE et al. 1983; KUZIO et al. 1984). These sequences have been associated with RNA polymerase II transcribed genes.

The identification and mapping of viral-encoded mRNAs has been achieved by various techniques including: hybridization selection of mRNA using purified vDNA (SMITH et al. 1982; ROHEL et al. 1984) or cloned restriction fragments

of vDNA (ESCHE et al. 1982; SMITH et al. 1983; LUBBERT and DOERFLER 1984a); purification of radiolabeled intracellular RNA and subsequent hybridization to vDNA (ERLANDSON and CARSTENS 1983); northern blot analysis of RNA using cloned fragments of vDNA as probes (ROHEL and FAULKNER 1984; ERLANDSON et al. 1985); and the use of cDNA clones as probes for specific mRNAs (ADANG and MILLER 1982). By following the synthesis of virus-specific RNA at intervals postinfection, a complex pattern of virus transcription has emerged (ROHEL and FAULKNER 1984; ERLANDSON et al. 1985). The overall pattern of virus transcription has been studied by hybridizing ^{32}P-labeled RNA (extracted at time points after infection) with vDNA using a dot-blot procedure (ERLANDSON and CARSTENS 1983). The results indicate that there are two major phases of transcription. The first, which precedes the initiation of vDNA replication, begins within 1 h of infection and continues for about 6 h. The early period encompasses several distinct stages of transcription involving changes in either the recognition of promoter regions or polyadenylation sites of transcripts with the same major body of RNA (LUBBERT and DOERFLER 1984b; FRIESEN and MILLER 1985). The early phase is followed by a dramatic quantitative increase in the level of transcription. In this late stage, transcription appears to continue until the infected cells lyse (ROHEL and FAULKNER 1984).

Two approaches have been used to delineate the location of transcriptionally active regions on the physical map during the replication cycle of AcMNPV. In the first, both total cytoplasmic and poly (A)+RNA, labeled in cell cultures and harvested at intervals postinfection, were hybridized to Southern blots of cloned fragments of AcMNPV DNA (VLAK and VAN DER KROL 1982; ERLANDSON and CARSTENS 1983). Eight specific regions were identified which synthesized major virus transcripts as early as 2.5 h postinfection. There was no apparent clustering of early regions, since the early transcripts hybridized to almost all loci on the genome (ERLANDSON and CARSTENS 1983). However, it was possible to distinguish regions of early transcription based on the relative intensities of hybridization to the DNA fragments, and to estimate the level of transcription. There was an increase in the level of transcription from other regions of the genome which coincided with the initiation of vDNA synthesis at 6 h postinfection, suggesting that expression of early transcripts was necessary before additional regions of the genome could be transcribed. A requirement for early transcription is also suggested by the analysis of virus transcription with a temperature-sensitive mutant, ts8, defective in the synthesis of DNA at the nonpermissive temperature (ERLANDSON et al. 1985). Transcripts normally seen late after infection were absent in infected cells at the restricted temperature. Some overlapping early transcripts of increasing size which hybridized with AcMNPV *Eco*RI J and *Eco*RI L fragments were also detected. This suggests that the defect in ts8 probably does not affect the transcription patterns observed in the alpha and beta phases of polypeptide synthesis (KELLY and LESCOTT 1981; see Sect. 7).

A second approach to study the overall transcription pattern of AcMNPV has used northern blot analysis of cytoplasmic or poly (A)+RNA harvested at increasing times postinfection (ROHEL and FAULKNER 1984; ERLANDSON et al. 1985). The blots were probed with labeled cloned vDNA restriction fragments