Virology 476 (2015) 257–263

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Baculoviruses and nucleosome management

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ARTICLE INFO

Article history:
Received 5 November 2014
Returned to author for revisions
9 December 2014
Accepted 10 December 2014
Available online 5 January 2015

Keywords:
Baculovirus
AcMNPV
Autographa californica M nucleopolyhedrovirus
Nucleosomes
Viral chromatin
Actin-containing chromatin modification complexes
SWI/SNF, INO80, TIP60
Actin
Topoisomerase 2
Negatively-supercoiled ds DNA

ABSTRACT

Negatively-supercoiled-ds DNA molecules, including the genomes of baculoviruses, spontaneously wrap around cores of histones to form nucleosomes when present within eukaryotic nuclei. Hence, nucleosome management should be essential for baculovirus genome replication and temporal regulation of transcription, but this has not been documented. Nucleosome mobilization is the dominion of ATP-dependent chromatin-remodeling complexes. SWI/SNF and INO80, two of the best-studied complexes, as well as chromatin modifier TIP60, all contain actin as a subunit. Retrospective analysis of results of AcMNPV time course experiments wherein actin polymerization was blocked by cytochalasin D drug treatment implicate actin-containing chromatin modifying complexes in decatenating baculovirus genomes, shutting down host transcription, and regulating late and very late phases of viral transcription. Moreover, virus-mediated nuclear localization of actin early during infection may contribute to nucleosome management.

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Introduction

The study of baculoviruses, pathogens of larval lepidopteran insects, has been justified mainly by aims of improving their abilities to serve humanity—as pesticides, expression vectors, vaccine platforms, drug therapy vectors and more (Moscardi, 1999; Chuang et al., 2007; Airenne et al., 2013; Fernandes et al., 2013; Assenberg et al., 2013; Contreras-Gómez et al., 2013; Grabherr and Ernst, 2013; Van Oers et al., 2014). Investment in the utility of baculoviruses as laboratory tools and industrial workhorses has overshadowed investment in basic studies on how these viruses actually function. *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is the type species of the Alphabaculovirus genus of *Baculoviridae* and is the most-studied of the baculoviruses (Herniou et al., 2011). AcMNPV arguably has received more attention than any other insect virus that is not pathogenic for humans. Even so, large gaps remain in

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http://dx.doi.org/10.1016/j.virol.2014.12.022

understanding the molecular strategies that AcMNPV and other baculoviruses use to reproduce and to make the cells they infect such powerful protein expression machines.

The current paradigm holds that baculovirus gene expression occurs in four temporal phases and is regulated primarily at the transcriptional level; that baculoviruses are the only nuclear replicating DNA viruses that use a combination of host and viral polymerases for viral gene transcription; that TATA boxes and CAGT motifs promote transcription during the early phases, and that ATAAG, GTAAG or TTAAG motifs promote transcription during the late phases (Rohrmann, 2013). Genome sequence has provided the basis for most baculovirus studies conducted during the last several decades and a great deal has been learned. But baculoviruses, like papillomaviruses and polyomaviruses of vertebrates, have circular, negatively-supercoiled, double-stranded (ds) DNA genomes, and any effect genome structure might have on regulation of transcription and replication of baculoviruses has yet to be explored. This omission in baculovirus lore may be attributable to the influence of the long-standing view that DNA is a passive polymer bullied by proteins that dictate their interactions (Cozzarelli et al., 2006). This view has changed, however. DNA topologists have unequivocally demonstrated that DNA structure has an active role in regulating biological processes (Liu et al., 2009; Fogg et al., 2012).

Two lessons from DNA topology have immediate relevance for baculoviruses: (1) covalently-closed circular (ccc) supercoiled DNA replication results in intertwined rings of DNA that are decatenated in eukaryotes by topoisomerase 2 (Topo 2) (Liu et al., 2009); and (2) negatively-supercoiled DNA immediately forms nucleosomes on entering host nuclei making nucleosome manipulation essential both for baculovirus replication and temporal regulation of transcription (Patterton and von Holt, 1993; Baranello et al., 2012). To date, these principles have not been integrated into the baculovirus paradigm even though evidence that baculovirus genomic DNA is negatively supercoiled has existed for decades.

Models of negatively supercoiled ds DNA behavior abound (Witz and Stasiak, 2010). Chromosomal and plasmid DNA molecules are negatively supercoiled in all bacteria, except for in extreme thermophiles, giving DNA topologists and microbiologists ample material with which to study the properties of negatively supercoiled DNA in biological systems (Schvartzman et al., 2013). Current evidence suggests that because torsional tension diminishes the DNA helicity and facilitates strand separation, negative supercoiling assists in DNA replication, transcription and aids DNA topoisomerases in decatenating DNA. In short, negative supercoiling has emerged as a major factor in the governance of biological activities (Witz and Stasiak, 2010; Schvartzman et al., 2013).

The AcMNPV genome is negatively supercoiled and forms nucleosomes

Summers and Anderson (1973) used cesium chloride density gradient centrifugation to show that baculovirus genomes consist of ccc, supercoiled ds DNA. That the supercoiling is negative was demonstrated by the rapid inactivation of baculoviruses by exposure to psoralens in 365 nm light (Hartig et al., 1992; Weightman and Banks, 1999). Psoralens are bi-functional photoreactive molecules that preferentially intercalate into negatively supercoiled ds DNA and photoreact at 365 nm, forming cross-links between the apposing strands (Naughton et al., 2013; Kouzine et al., 2014).

Wilson and Miller (1986) noted that parental AcMNPV DNA formed chromatin-like structures within 2 h of uncoating within the nucleus, consistent with the properties of a negatively supercoiled ds DNA molecule (Baranello et al., 2012). Additionally, treatment of DNA from infected cells with micrococcal nuclease resulted in monosome-sized fragments that contained both cellular and viral DNA. Extending these findings, Wilson and Price (1988) observed that infection resulted in increased association of cellular histones with the nuclear matrix, and that progeny AcMNPV DNA incorporated histones prior to 24 hpi, all supporting the idea that AcMNPV DNA forms nucleosomes in the nucleus.

Nucleosomes consist of 147 bp of DNA wrapped around a core histone octamer, and these structures are spaced every 10 to 50 bp in chromatin (Moshkin et al., 2012). Not surprisingly, they can impede access of DNA binding proteins to their target sequences, thereby preventing both transcription and replication. There is no basal transcription on cellular chromatin templates in vitro without factors mediating nucleosome repositioning or ejection (Moshkin et al., 2012). Transcription takes place when AcMNPV chromatin templates are transfected into susceptible host cells, however, showing the immediate early genes are open for business (Burand et al., 1980). Chromatin-modifiers present within the transfected cells may have a role in immediate early gene transcription, but negative supercoiling allows access to binding sites not available in positively supercoiled or even neutral DNA (Baranello et al., 2012). Accordingly, transfection studies have shown that baculovirus genome replication is 4 times more efficient when the template is supercoiled than when it is nicked, and 15–150 times more efficient than when it is linearized (Burand et al., 1980; Kitts et al., 1990).

If negative supercoiling enables transcription of AcMNPV immediate early genes and thereby offers a way out of nucleosomal constraints, then subsequent viral transcriptional programs (delayed early, late and very late) would be expected to depend on virus-encoded factors capable of modulating chromosome-remodeling systems.

Moving nucleosomes

Nucleosome mobilization is the specialty of ATP-dependent chromatin remodeling complexes (Ho and Crabtree, 2010). Each complex is named after the primary ATPase that does the work. INO80 and SWI/SNF comprise two of the four main families of these complexes. They are evolutionarily conserved from yeast through vertebrates. These complexes are thought to have played a major role in the evolution of multicellularity and the demand for tissue-specific and developmental-stage-specific expression of genes (Ho and Crabtree, 2010; Moshkin et al., 2012).

The ATP-dependent chromatin remodeling complexes consist of 8–15 combinatorially assembled components with a core group of essential subunits surrounded by others that, in vertebrates, at least, switch in and out during specific stages of development and differentiation. All of the complexes are thought to have specialized, non-redundant roles in development. Some of the complexes are cell-type and developmental-stage specific (Ho and Crabtree, 2010). Use of a combinatorial complex for chromatin remodeling during development and differentiation enables the formation of hundreds of complexes for diverse gene expression patterns (Moshkin et al., 2012).

This wide selection of complexes could be vulnerable to interception and repurposing by negatively supercoiled viruses with nucleosome management issues of their own. Subunit modification or faux subunit synthesis could be used to help recruit the ATP-dependent complexes to viral chromatin for specific tasks. In this regard, it is worth noting that all group I NPVs have orthologs of SNF2 (Katsuma et al., 2008). SNF2 ATPases are the major ATPases in many SWI/SNF and INO80 chromatin-remodeling complexes. These ATPases have active roles in both substrate recognition and catalytic activities (Morrison and Shen, 2009). Moreover, during BmNPV infection of Bm5 cells, genes related to organism development are up-regulated differentially throughout early, late and very late viral gene expression (Xue et al., 2012).

Actin-containing chromatin remodeling complexes

Actin is an abundant, conserved, 42 kDa ATPase that is regulated by a plethora of actin-binding proteins for many different cytoplasmic and nuclear functions (Krauss et al., 2003; Cisterna et al., 2009; Visa and Percipalle, 2010; Simon and Wilson, 2011; Rajakylä and Vartiainen, 2014). Cytoplasmic functions of actin inevitably depend on its ability to polymerize. Interestingly, this activity may be dispensable for some nuclear functions (Posern et al., 2002; Johnson et al., 2013; Kapoor and Shen, 2014).