The Protamine-like DNA-binding Protein P6.9 Epigenetically Up-regulates *Autographa californica* Multiple Nucleopolyhedrovirus Gene Transcription in the Late Infection Phase

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**Abstract**: Protamines are a group of highly basic proteins first discovered in spermatozoon that allow for denser packaging of DNA than histones and will result in down-regulation of gene transcription[1]. It is well recognized that the *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) encodes P6.9, a protamine-like protein that forms the viral subnucleosome through binding to the viral genome[29]. Previous research demonstrates that P6.9 is essential for viral nucleocapsid assembly, while it has no influence on viral genome replication[31]. In the present study, the role of P6.9 in viral gene transcription regulation is characterized. In contrast to protamines or other protamine-like proteins that usually down-regulate gene transcription, P6.9 appears to up-regulate viral gene transcription at 12-24 hours post infection (hpi), whereas it is non-essential for the basal level of viral gene transcription. Fluorescence microscopy reveals the P6.9's co-localization with DNA is temporally and spatially synchronized with P6.9's impact on viral gene transcription, indicating the P6.9-DNA association contributes to transcription regulation. Chromatin fractionation assay further reveals an unexpected co-existence of P6.9 and host RNA polymerase II in the same transcriptionally active chromatin fraction at 24 hpi, which may probably contribute to viral gene transcription up-regulation in the late infection phase.

**Key words**: Epigenetics; AcMNPV; P6.9; Protamine; Subnucleosome

In the eukaryotic nucleus, the genomic DNA and histones are packaged and organized into a nucleoprotein complex called “chromatin”. The fundamental packaging unit of chromatin is the nucleosome, an octamer of histones around which 147 base pairs (bp) of DNA is wrapped twice[21]. The
linker histone H1 interacts with both the nucleosome core and the linker DNA, and promotes higher-order folding and compaction of chromatin (reviewed in [24]). Besides assembling DNA into chromatin to form higher-order structures in the eukaryotic nucleus, the histones also play an active role in the regulation of gene transcription through establishing a dynamic molecular interface for transcription factors and RNA polymerases to bind to DNA sequences [27].

Protamines are a group of relatively small (4.0-12.0 kDa) and structurally heterogeneous proteins. A chemical definition of the protamine can be deduced from its sequence composition of ≥40% arginine with a few or no lysine [18]. Protamines reportedly serve as functional counterparts of histones, and the remodeling from a histone- to a protamine-based chromatin will usually result in higher condensation of genomic DNA and gene transcription down-regulation (reviewed in [3]).

Besides cellular chromatin, viral chromatin also exists and plays an important role in the life cycle of many viruses (reviewed in [19]). Viruses such as simian virus 40 (SV40) and polyomavirus which use host enzymes to replicate their DNA tend to use host histones to package viral genomic DNA into virions [4]. Alternatively, for viruses such as adenoviruses using virus-encoded replication machinery, they tend to form viral chromatin via virus-encoded histone-like proteins [28].

Baculoviruses are large double-stranded DNA viruses and among them, *Autographa californica* multicapsid nucleopolyhedrovirus (*AcMNPV*) is one of the most extensively studied prototypes. The *AcMNPV* basic DNA-binding protein P6.9 exhibits a protamine-like amino acid composition (44% arginine and no lysine) [29]. By 10 hours post infection (hpi), P6.9 becomes associated with the viral DNA [34]. By 24 hpi, the nucleosome-like structures are completely substituted by subnucleosome-sized DNA fragments of 120 and 90 bp chromatin structure containing exclusively viral DNA [33]. As a protamine-like chromosomal protein, P6.9 was supposed to form a higher condensed chromatin and down-regulate *AcMNPV* gene transcription. However, Wilson *et al* provided evidence that the subnucleosome-sized *AcMNPV* chromatin is sensitive to micrococcal nuclease digestion, which is correlated to transcriptional activity [33]. This unexpected phenotype implies that P6.9’s role in regulation of viral gene transcription is probably distinct from the protamines or other protamine-like proteins. However, the detailed role of P6.9 in regulation of *AcMNPV* gene transcription remains unknown.

Previous research demonstrates that P6.9 is essential for viral nucleocapsid assembly, but it has no influence on viral genome replication [31]. In the present study, the epigenetic role of P6.9 in regulation of *AcMNPV* gene transcription was characterized. We found that P6.9 as a protamine-like chromosomal protein up-regulates viral gene transcription at 12-24 hpi, which is opposite to the protamines or other protamine-like proteins that usually down-regulate gene transcription.

**MATERIALS AND METHODS**

**Cell culture and virus**

Sf9 cells were cultured at 27°C in Grace’s media containing 10% fetal bovine serum (FBS) (Gibco). *AcMNPV* recombinant bacmids were derived from bMON14272 (Invitrogen) [20], and propagated in...