Virology 436 (2013) 67–74

Contents lists available at SciVerse ScienceDirect

Virology

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

Host RNA polymerase inhibitors encoded by ϕKMV-like phages of *pseudomonas*

Evgeny Klimuk [a,b], Natalia Akulenko [a], Kira S. Makarova [c], Pieter-Jan Ceyssens [d], Ivan Volchenkov [b], Rob Lavigne [d], Konstantin Severinov [a,b,e,*]

[a] *Institutes of Molecular Genetics and Gene Biology of the Russian Academy of Sciences, Moscow, Russia*
[b] *Evrogen JSC, Miklukho-Maklaya 16/10, 117997 Moscow, Russia*
[c] *National Center for Biotechnology Information, NLM, National Institutes of Health, Bethesda, MD 20894, USA*
[d] *Laboratory of Gene Technology, Biosystems Department, KU Leuven, Belgium*
[e] *Waksman Institute for Microbiology and Department of Molecular Biology and Biochemistry, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, USA*

ARTICLE INFO

Article history:
Received 17 August 2012
Returned to author for revisions
27 September 2012
Accepted 11 October 2012
Available online 3 November 2012

Keywords:
Bacteriophages T7 and ϕKMV
Pseudomonas
RNA polymerase
RNA polymerase inhibitor

ABSTRACT

Escherichia coli bacteriophage T7 is a founding member of a large clade of podoviruses encoding a single-subunit RNA polymerase (RNAP). Phages of the family rely on host RNAP for transcription of early viral genes; viral RNAP transcribes non-early viral genes. T7 and its close relatives encode an inhibitor of host RNAP, the gp2 protein. Gp2 is essential for phage development and ensures that host RNAP does not interfere with viral RNAP transcription at late stages of infection. Here, we identify host RNAP inhibitors encoded by a subset of T7 clade phages related to ϕKMV phage of *Pseudomonas aeruginosa*. We demonstrate that these proteins are functionally identical to T7 gp2 *in vivo* and *in vitro*. The ability of some *Pseudomonas* phage gp2-like proteins to inhibit RNAP is modulated by N-terminal domains, which are absent from the T7 phage homolog. This finding indicates that *Pseudomonas* phages may use external or internal cues to initiate inhibition of host RNAP transcription and that gp2-like proteins from these phages may be receptors of these cues.

Introduction

During the infection of *Escherichia coli* by bacteriophage T7, only ~2% of phage genome corresponding to genetically defined left end enters the infected cell immediately after the phage attachment (Garcia and Molineux, 1995; Kemp et al., 2005). The remaining DNA is brought in by transcription, first by *E. coli* and then by T7 RNAP (Kemp et al., 2004). The leading part of the genome contains strong early promoters recognized by the host σ^{70} RNAP holoenzyme ($E\sigma^{70}$). Transcription by $E\sigma^{70}$ from the early promoters causes at least 7 kbp of the ~40 kbp T7 genome to enter the bacterial cell (Kemp et al., 2004). The mRNAs produced from these promoters code for several phage proteins involved in the shut-off of host defenses, such as gp0.3, an antirestriction protein (Studier, 1975; Mark and Studier, 1981; Walkinshaw et al., 2002), and gp0.7, a protein kinase that phosphorylates the β' subunit of host RNAP and affects its termination properties (Severinova and Severinov, 2006). *E. coli* RNAP also transcribes gene 1, which encodes a single-subunit, rifampicin-resistant viral RNAP. The latter enzyme normally completes the genome internalization process by transcription from its own promoters, which are located throughout the genome (Garcia and Molineux, 1999).

T7 RNAP transcribes the middle and late genes of the phage (Fig. 1). Genes from the former group mostly code for proteins involved in phage DNA replication. Genes from the latter group encode structural proteins of the T7 virion. The product of phage middle gene 2 is a potent inhibitor of host RNAP (Hesselbach and Nakada, 1977). Gp2 is a 7 kDa protein that binds to the host RNAP β' subunit downstream jaw domain and prevents open promoter complex formation by $E\sigma^{70}$ (Nechaev and Severinov, 1999). Gp2 has no effect on transcription by T7 RNAP (LeClerc and Richardson, 1979).

T7 gene 2 is an essential gene. Analysis of infection of non-permissive cells by T7 gene 2 amber ($T7^{2am}$) mutants reveals that the infection is blocked at the stage of packaging of concatemeric viral DNA into virion heads (LeClerc and Richardson, 1979). The absence of gp2 can be complemented by treating $T7^{2am}$–infected cells with rifampicin, an inhibitor of host RNAP, after the onset of middle gene transcription (Ontell and Nakada, 1980; Mooney et al., 1980). The result thus suggests that the only essential function of gp2 is the inhibition of host RNAP transcription, which apparently becomes deleterious late in infection. The exact mechanism by which host RNAP interferes with phage development is not known,

* Corresponding author at: Waksman Institute, 190 Frelinghuysen Road, Piscataway, NJ 08854, USA. Fax: +1 732 445 5735.
E-mail address: severik@waksman.rutgers.edu (K. Severinov).

0042-6822/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2012.10.021

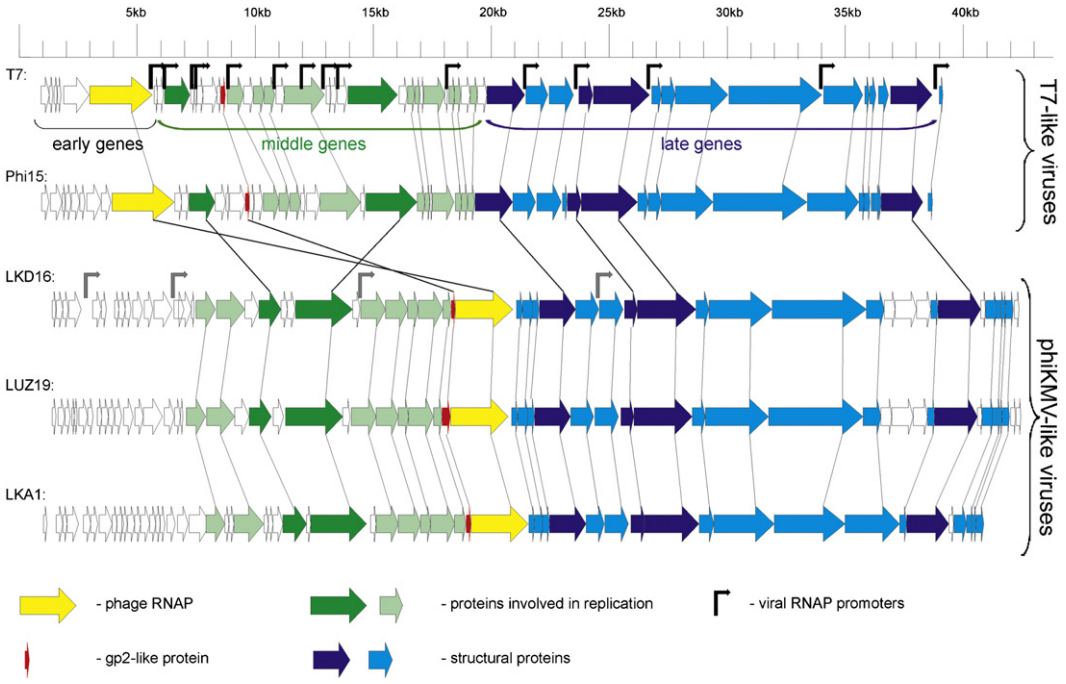

Fig. 1. Comparison of T7- and ϕKMV-subgroup phage genomes.The genome of T7 phage is schematically presented at the top and aligned with a phi15 (a T7-subgroup *Pseudomonas* phage) and ϕKMV-subgroup *Pseudomonas* phages LKD16, LUZ19, and LKA1. In T7, genes belonging to different temporal expression classes (early, middle, and late) are indicated. Some replication (dark green) and structural (dark blue) genes are homologous in all five genomes, as are the viral RNAP genes (yellow). Host RNAP inhibitor genes are shown in red. Genes indicated by lighter shaded of green or blue are conserved within T7- of ϕKMV-subgroups only. Rightward arrows indicate viral RNAP promoters, known (for T7) or predicted[18] (for LKD16).

however, disruption of one of the early promoters, A3, makes gp2 dispensable (Qimron et al., 2008; Savalia et al., 2010). It has been proposed that when the slower moving host RNAP transcribes into extended middle and late gene clusters of the phage, it becomes an obstacle to the much faster viral RNAP, causing the latter to stall and recruit phage DNA packaging/concatemer cleavage machinery at aberrant sites, leading to generation of less-than-unit length non-functional phage genomes (Qimron et al., 2008).

T7 is a founding member of a very large clade of phages of the *Podoviridae* family (Molineux, 2006). Many members of the family (sometimes referred to as "the T7 supergroup" (Hardies et al., 2003) and now formally classified as the *Autographivirinae* subfamily within the ICTV IXth report) encode a single-subunit viral RNAP. While some phages, belonging to the T7-subgroup, are very similar to T7 in their genomic organization and likely share a common strategy of infection, others are quite different. In particular, a clearly distinct subgroup, named after the *Pseudomonas aeruginosa* ϕKMV phage (Lavigne et al., 2003; Ceyssens et al., 2006) was described. Phages from this group infect bacteria of the *Pseudomonas* genus and differ from T7 and its close relatives in several respects, most obviously in the position of the viral RNAP gene. In ϕKMV-like phages, the viral RNAP gene is located close to the middle of the genome, between the replication and structural gene clusters (Fig. 1). Therefore, the process of phage DNA injection in host cell, and the temporal control of gene expression of ϕKMV-like phages must differ significantly from those operating in the T7 subgroup. In fact, no homologs of T7 gene 2 are listed in available annotations of ϕKMV-related phage genomes (Lavigne et al., 2003; Ceyssens et al., 2006; Kulakov et al., 2009). However, since ϕKMV-like phages probably rely on viral RNAP transcription for expression of at least their structural genes, one can hypothesize that a host RNAP inhibitor(s) either analogous or homologous to gp2 proteins encoded by T7 subgroup phages may be also encoded by ϕKMV-related phages. In this paper we identify host RNAP inhibitors encoded by ϕKMV-like phages and show that these proteins share a common RNAP binding site with T7 gp2 and can functionally substitute for T7 gp2 during the infection. We propose that most, or, perhaps, all phages encoding their own RNAP, also encode inhibitors of host RNAP that ensure orchestrated transcription of phage genes during the infection.

Results

Identification of phage proteins interacting with pseudomonas RNAP in cells infected with ϕKMV-like phages

Co-affinity purification coupled with mass spectrometric identification is a powerful method to identify unknown phage proteins binding to host RNAP (Savalia et al., 2008; Westblade et al., 2008). To apply this approach to ϕKMV-like phages, we constructed a *P. aeruginosa rpoC::PrA* strain encoding a Protein A tag attached to the C-terminal end of the RNAP β' subunit. The strain was viable and indistinguishable from parental wild-type strain at laboratory conditions. The strain was infected with ϕKMV-like phages LKA1 or LUZ19 (Ceyssens et al., 2006), cells were harvested 10 min post-infection and processed for affinity purification of RNAP. Mass-spectrometric identification revealed the presence of Eσ^{70} components (RNAP α, β, β', ω, and σ^{70}) and host transcription factor NusA. In addition peptides from phage LKA1 protein gp36 and LUZ 19 gp25.1 were also detected (Supplementary Tables 1 and 2), indicating that these proteins might interact with host RNAP in infected cells.

Bioinformatic prediction of ϕKMV-like phage proteins similar to T7 gp2

Phage LKA1 protein gp36 and phage LUZ19 gp25.1 are small proteins that, according to available annotations, have no similarity to proteins of known function in public databases. C-terminal parts of both proteins are similar to each other and both proteins are encoded by genes located immediately