Virology 468-470 (2014) 226–237

Contents lists available at ScienceDirect

Virology

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

Complementation for an essential ancillary non-structural protein function across parvovirus genera

Ivailo S. Mihaylov [a], Susan F. Cotmore [a], Peter Tattersall [a,b,*]

[a] Department of Laboratory Medicine, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510, USA
[b] Department of Genetics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510, USA

ARTICLE INFO

Article history:
Received 7 June 2014
Returned to author for revisions
9 July 2014
Accepted 21 July 2014

Keywords:
Minute virus of mice (MVM)
Human bocavirus 1 (HBoV1)
Parvovirus
Non-structural proteins
NS2
NP1
Functional complementation
DNA replication block

ABSTRACT

Parvoviruses encode a small number of ancillary proteins that differ substantially between genera. Within the genus *Protoparvovirus*, minute virus of mice (MVM) encodes three isoforms of its ancillary protein NS2, while human bocavirus 1 (HBoV1), in the genus *Bocaparvovirus*, encodes an NP1 protein that is unrelated in primary sequence to MVM NS2. To search for functional overlap between NS2 and NP1, we generated murine A9 cell populations that inducibly express HBoV1 NP1. These were used to test whether NP1 expression could complement specific defects resulting from depletion of MVM NS2 isoforms. NP1 induction had little impact on cell viability or cell cycle progression in uninfected cells, and was unable to complement late defects in MVM virion production associated with low NS2 levels. However, NP1 did relocate to MVM replication centers, and supports both the normal expansion of these foci and overcomes the early paralysis of DNA replication in NS2-null infections.

© 2014 Elsevier Inc. All rights reserved.

Introduction

Viruses in the family *Parvoviridae* have a linear single-stranded DNA genome of around 5 kb, with small self-priming hairpin telomeres. Their constrained size leads them to be genetically compact, using from one to three transcriptional promoters and one or two polyadenylation signals to coordinate expression from two genes clusters; a nonstructural (NS or *rep*) gene that encodes the viral replication initiator protein (NS1 or rep), and a structural (VP or *cap*) gene that encodes two or more size variants of a single capsid protein. In addition, all viruses encode a few small ancillary proteins with disparate structures and functions, which are variably disposed throughout the genome (reviewed in Agbandje-McKenna and Kleinschmidt (2011), Berns and Parrish (2013), Cotmore and Tattersall (2013), and Halder et al. (2012)). These ancillary species are typically conserved between viruses within each genus, but are widely divergent between genera as illustrated for minute virus of mice (MVM), a member of genus *Protoparvovirus*, and for human bocavirus-1 (HBoV-1), from the genus *Bocaparvovirus*, in Fig. 1A and B respectively. Genus names used throughout this paper reflect a recent taxonomic revision in which parvoviruses that infect vertebrate hosts and together constitute the subfamily *Parvovirinae* are now divided between eight genera, all of which carry the infix "parvo" in their genus names, to clarify their family affiliation as discussed in Cotmore et al. (2013), and posted online at: http://talk.ictvonline.org/files/ictv_official_taxonomy_updates_since_the_8th_report/m/vertebrate-official/4844.aspx (Taxonomy of the family Parvoviridae).

Due to their genetic minimalism, parvovirus DNA replication mechanisms rely predominantly on the synthetic machinery of their host cell, assisted and organized by the NS1 protein, while the ancillary proteins fulfill multiple variable functions during the viral life cycle, often serving, at least in part, to modulate the host environment. In the current study we asked to what extent replication defects that result from loss of the NS2 family of ancillary factors encoded by MVM, can be complemented in trans by expression of the very different HBoV1 NP1 protein. Viruses in these two genera are relatively close phylogenetically, and share the important characteristic of being heterotelomeric, meaning that the hairpin telomeres at the two ends of the genome are very different from each other. In MVM these disparate structures are known to be processed by different mechanisms and at different rates, adding a layer of complexity to the replication process that is not seen for homotelomeric parvoviruses, such as those from the *Dependoparvovirus* or *Erythroparvovirus* genera (Cotmore and Tattersall, 2013). Nevertheless, the analogous terminal hairpin

* Corresponding author at: Department of Laboratory Medicine, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510, USA.
Fax: +1 203 737 4326.
E-mail address: peter.tattersall@yale.edu (P. Tattersall).

http://dx.doi.org/10.1016/j.virol.2014.07.043
0042-6822/© 2014 Elsevier Inc. All rights reserved.

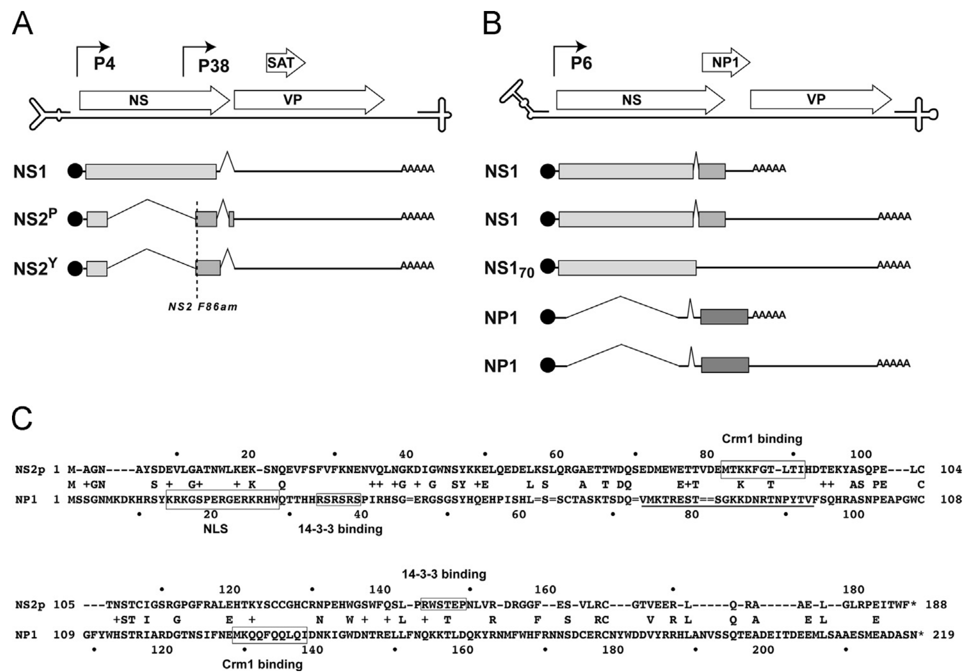

Fig. 1. Comparison of the coding strategies and sequences of MVM NS2 and HBoV1 NP1 proteins. Panels A and B: The coding sequences of (A) MVM and (B) HBoV1 proteins and positions of the viral promoters are aligned above line diagrams of the single-stranded viral genomes, which terminate in folded hairpin structures, shown expanded ~20-fold in scale relative to the coding regions. Below these, NS protein-encoding transcripts are indicated by lines. These express blocks of protein sequence encoded in separate reading frames, indicated by pale, medium and dark gray blocks. AAAAA=polyadenylation sites. "NS2-F86am" marks the position of a termination codon introduced into MVM that prevents stable expression of all NS2-encoding transcripts. Panel C: The protein sequence of NS2P aligned above that of NP1. Positions of actual NS2 Crm1 and 14-3-3 binding sites and hypothetical NP1 binding sites for these proteins are shown boxed, together with the functional NP1 NLS sequence.

sequences of MVM and HBoV1 are very different from each other (Huang et al., 2012), and this is mirrored by their widely disparate ancillary proteins. While both NP1 and NS2 are known to be required for the productive replication of their respective viruses (Chen et al., 2010; Cotmore et al., 1997; Lederman et al., 1984; Naeger et al., 1990, 1993; Sukhu et al., 2013), they are encoded in different ways, and the proteins show no apparent protein sequence similarity to each other (Fig. 1C), have very different turn-over rates, and predominantly occupy different cellular compartments.

As seen in Fig. 1A, MVM encodes two major forms of the ~25 kDa NS2 protein, called NS2P and NS2Y, generated by alternative splicing at a small centrally-positioned intron, which differ only in their extreme C-terminal hexapeptides (Morgan and Ward, 1986; Cotmore and Tattersall, 1990). While NS2 proteins are typically dispensable for productive viral replication in transformed human cell lines (Naeger et al., 1990), they are absolutely required in cells of the virus's normal murine host. These proteins all share a common *N* terminal domain of 84 amino acids with NS1, but are then spliced into a different open reading frame, as shown in Fig. 1A. NS2P and NS2Y "isoforms" accumulate at a ratio of approximately 5:1 during infection, reflecting the stoichiometry of splice donor and acceptor site usage at this intron. NS2 isoforms are the most abundantly-expressed viral proteins during the first few hours after the cell enters S-phase (Ruiz et al., 2006), but both forms are subject to rapid proteosomal degradation (Miller and Pintel, 2001), resulting in a half-life of around 1 h (Cotmore and Tattersall, 1990).

Mutant viruses that fail to stably express NS2 show a severe defect in viral DNA replication that restricts the accumulation of duplex replicative-form (RF) DNA intermediates to ~5% of wildtype levels. While the role of the NS2Y isoform remains uncertain, since mutant viruses that fail to express this species replicate like wildtype virus, mutants that lack NS2P initiate infection efficiently in A9 cells but viral DNA replication aborts early in the duplex amplification phase. This phenotype can be tracked by immunofluorescence microscopy, where it is seen to manifest between 6 and 12 h into S-phase as a profound block to the normal progressive expansion of viral replication centers (Ruiz et al., 2006), known as autonomous parvovirus-associated replication (APAR) bodies (Cziepluch et al., 2000). However, these structures apparently continue to sequester the same broad range of cellular replication and DNA damage-response proteins that accumulate during the early stages of wildtype infection (Ruiz et al., 2011), and even low level expression of NS2P can drive infected cells through the block, suggesting that it involves a small number of NS2 targets or an enzymatic process. In this paper we refer to the ability to overcome this early duplex amplification block as the "critical early function" of NS2.

However, mutants that are able to express low levels of NS2P are still severely impaired in their ability to expand through murine cells, requiring progressively higher, doses of NS2 to maximize the production of progeny virions. The roles played by NS2 in this late "progeny function" are complex, since it is required for both efficient capsid assembly, which is a pre-requisite for virion production, and for early virion release prior to cell lysis, which greatly enhances the rate at which virus spreads between cells. During infection all NS2 species appear predominantly cytoplasmic, in part due to interactions with the nuclear export factor, Crm1, as illustrated by the fact that leptomycin *B*, a Crm1 inhibitor, allows NS2 to accumulate in the nucleus (Bodendorf et al., 1999). In vitro NS2 binds with high "supraphysiological" affinity to Crm1 (Bodendorf et al., 1999; Engelsma et al., 2008; Ohshima et al., 1999), but mutations that modify its Crm1-binding site (Miller and Pintel, 2002), or reduce it to one of physiologically-normal affinity (Eichwald et al., 2002; Engelsma et al., 2008), substantially impair progeny virus production. This implicates the NS2:Crm1 interaction in a late step(s) in virion production or in their subsequent export, perhaps via assembly of a tripartite (Crm1:NS2:virion) nuclear export complex, although such

Download English Version:

https://daneshyari.com/en/article/6139741

Download Persian Version:

https://daneshyari.com/article/6139741

Daneshyari.com