Available online at www.sciencedirect.com

SCIENCE DIRECT

Virology 332 (2005) 225–234

VIROLOGY

www.elsevier.com/locate/yviro

The polymerase (L) protein of rinderpest virus interacts with the host cell protein striatin

Katrina Sleeman[1], Michael D. Baron*

Molecular Virology and Parasitology, Institute for Animal Health, Ash Road, Pirbright, Surrey GU24 0NF, UK

Received 21 September 2004; returned to author for revision 22 October 2004; accepted 12 November 2004
Available online 15 December 2004

Abstract

Rinderpest virus (RPV) is a morbillivirus that causes a highly contagious disease affecting members of the order *Artiodactyla*. The viral L protein is the catalytic subunit of the RNA-dependent RNA polymerase. To search for host cell proteins with which L interacts, a library screen was performed using the yeast two-hybrid system. Several host cell proteins were recovered from the library screen as putative L-interactors; one of these was identified as striatin. A direct interaction between RPV L and striatin was confirmed using both co-immunoprecipitation assays and co-localisation studies using confocal microscopy. Striatin was also shown to co-localise with the RPV L protein in infected cells. The L proteins of morbilliviruses consist of three long highly conserved domains separated by short unconserved stretches of amino acids. The L domain with which striatin interacts was investigated by co-immunoprecipitation and striatin was shown to interact primarily with the central conserved domain.

Keywords: Rinderpest; Polymerase; Morbillivirus; Yeast two hybrid; Striatin

Introduction

Rinderpest is a highly contagious disease of ancient Asiatic origin that affects even-toed ungulates of the order *Artiodactyla*, most commonly cattle and buffalo. The disease is currently targeted for global eradication by 2010 (Anderson et al., 1996; Reid, 1981). If this goal is achieved, then it will be the first time that an animal disease will have been eliminated worldwide and, after smallpox, the second disease eradicated in history. The causative agent of this economically important disease is *Rinderpest virus* (RPV). RPV, which is closely related to the human *Measles virus* (MeV), is a member of the genus *Morbillivirus*, family *Paramyxoviridae* in the order *Mononegavirales* (Pringle, 1991; Schneemann et al., 1995).

The RNA-dependent RNA polymerase of monopartite negative-strand RNA viruses, such as RPV, consists of two virus-encoded subunits, the phosphoprotein (P) and the large (L) protein, the latter being the largest of the virus-encoded proteins. The paramyxovirus L protein is approximately 240 kDa in size and is the least abundant component in the viral nucleocapsid, with approximately 50 copies per virion (Lamb et al., 1976), correlating with the fact that it is encoded by the gene most distal from the viral genome promoter. The L protein is thought to act as a multifunctional enzyme, responsible for all catalytic activities necessary for viral RNA synthesis (Horikami et al., 1994), including initiation, elongation, termination, capping, methylation, and polyadenylation. Studies with the rhabdovirus *Vesicular stomatitis virus* (VSV) and the respirovirus *Sendai virus* (SeV) have confirmed that polyadenylation, methyltransferase, capping and kinase activities are associated with the L protein (Einberger et al., 1990; Gupta et al., 2002; Hammond et al., 1992; Hercyk et al., 1988; Horikami and Moyer, 1982; Hunt and Hutchinson, 1993). Formation of a complex between L and P is vital for these processes

* Corresponding author. Fax: +44 1483 232448.
E-mail address: michael.baron@bbsrc.ac.uk (M.D. Baron).
[1] Present address: Measles Virus Section, Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA.

0042-6822/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2004.11.020

(Hamaguchi et al., 1983). Furthermore, a 2′-*O*-ribose (cap 1) methyltransferase domain has recently been identified in the L protein of all mononegavirales (Bujnicki and Rychlewski, 2002; Ferron et al., 2002).

Building on previous observations (Barik et al., 1990; Feldhaus and Lesnaw, 1988), a sequence alignment and comparison study of L proteins of five monopartite negative-strand RNA viruses within the paramyxovirus and rhabdovirus families revealed highly conserved amino acids clustered into six domains along the length of L, joined by variable regions (Poch et al., 1990). The enzymatic functions of the polymerase are thought to be located within the conserved domains of L, thereby resembling a "chain of enzymes". McIlhatton et al. (1997) examined the sequences of the L proteins of morbilliviruses and noted that, for this genus, there were in fact only two small regions of poorly conserved sequence along the whole length of L, which separated three highly conserved domains. These two regions were referred to as "hinges" and, like the variable regions located between Poch's six domains, were thought to be flexible and enable optimal cooperation between the conserved domains.

In addition to the viral proteins, several host cell proteins have been shown to be required for activity of polymerases of paramyxoviruses or rhabdoviruses. MeV, SeV, and VSV (New Jersey strain) have all been shown to require the host cell protein tubulin for viral RNA synthesis (Moyer et al., 1986, 1990). While both MeV L and VSV L have been shown to interact directly with tubulin, no such interaction was observed in similar studies using SeV L (Moyer et al., 1990). Actin is known to be required for the transcription of not only *Human parainfluenza virus 3* (hPIV-3) (De et al., 1993), but also *Human respiratory syncytial virus* (RSV) (Burke et al., 1998). More recent work with RSV has demonstrated that another host cellular protein, profilin, is required for the optimal transcription of RSV (Burke et al., 2000), but this function of profilin required the presence of actin.

In addition to binding tubulin, the L protein of the prototype of nonsegmented negative strand RNA viruses, VSV, has recently been found to bind other host cell proteins including translation elongation factor-1α, an association that is required for its activity (Das et al., 1998) and heat shock protein 60 [Qanungo, 2004 #370]. In addition, VSV L protein appears to bind host cell guanylyl transferase (Gupta et al., 2002). The presence or absence of these proteins appears to differentiate mRNA transcribing forms from genome replicating forms of the virus polymerase [Qanungo, 2004 #370].

In addition to interactions of host cell proteins with the L subunit of the polymerase, direct binding of the dynein light chain to the P subunit has been shown in rabies virus [Poisson, 2001 #303] and of heat shock protein 72 to the nucleocapsid protein of MeV [Zhang, 2002 #369]. As we are interested in the interactions of RPV with its host cell, we wish to investigate whether any of the proteins of the viral replication complex (viral ribonucleoprotein) interact specifically with host cell proteins. We have used the yeast two-hybrid system (Y2HS) to identify novel protein binding partners of the L protein and confirmed that interaction by a variety of methods.

Results

Yeast two-hybrid system library screen

To enable the identification of novel host cell proteins that interact with the L protein of RPV, the yeast two-hybrid system (Fields and Song, 1989) was utilised. RPV is primarily a lymphotropic virus, infecting particularly cells of the macrophage/monocyte lineage (Rey Nores and McCullough, 1997). Since we had access to a well-characterised porcine macrophage cDNA library in a yeast two-hybrid vector and, although rinderpest is mainly thought of as a disease of bovines, it can productively infect domestic pigs (Govindarajan et al., 1996) and causes disease in warthogs and bush pigs in the wild (Anderson et al., 1996), a large-scale library screen was performed using this library and RPV L as the "bait" protein. Positive clones were selected by growth in the absence of histidine, and confirmed using two further interaction indicators, growth in the absence of adenine and expression of β-galactosidase. Several putative L interactors were recovered from this library screen. The library plasmids were recovered and put back into the yeast along with the L bait construct to confirm an interaction with L. The specificity of the isolated interaction was also confirmed by co-transforming the yeast with the recovered library plasmid and a bait construct containing a control gene (RPV C). Three of the recovered plasmids showed a strong interaction with L, but not with the C protein.

Identification of striatin

Each of these three recovered plasmids were then sequenced for identification purposes and the nucleotide sequences were submitted to an NCBI BLAST search to identify the putative L interacting partner. Only one of the constructs matched a known protein; the protein expressed from this clone was shown to be 92% similar to the amino terminus of the human and rat striatin proteins (Fig. 1). The interaction between L and striatin was further characterised by two additional techniques, co-immunoprecipitation and confocal microscopy.

Full-length L co-immunoprecipitates GST/striatin

To be sure that the observed interaction with the L protein was not an artefact of the yeast system, or the result of aberrant folding of the short section of striatin encoded by the cDNA insert, we obtained a full-length clone of striatin