Virology 485 (2015) 96–103

Contents lists available at ScienceDirect

Virology

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

Induction and characterization of a replication competent cervid endogenous gammaretrovirus (CrERV) from mule deer cells

Helena Fábryová [1,a], Tomáš Hron [a,1], Hana Kabíčková [b], Mary Poss [c], Daniel Elleder [a,*]

[a] Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, 14220 Prague, Czech Republic
[b] Military Health Institute, Department of Microbiology and Biological Research, 16001 Prague, Czech Republic
[c] Department of Biology, Center for Infectious Disease Dynamics, Pennsylvania State University, University Park, PA 16801, USA

ARTICLE INFO

Article history:
Received 9 April 2015
Returned to author for revisions
15 May 2015
Accepted 4 July 2015

Keywords:
Endogenous retrovirus
Xenotropism
Retrovirus interference

ABSTRACT

Endogenous retroviruses (ERVs) were acquired during evolution of their host organisms after infection and mendelian inheritance in the germline by their exogenous counterparts. The ERVs can spread in the host genome and in some cases they affect the host phenotype. The cervid endogenous gammaretrovirus (CrERV) is one of only a few well-defined examples of evolutionarily recent invasion of mammalian genome by retroviruses. Thousands of insertionally polymorphic CrERV integration sites have been detected in wild ranging mule deer (*Odocoileus hemionus*) host populations. Here, we describe for the first time induction of replication competent CrERV by cocultivation of deer and human cells. We characterize the physical properties and tropism of the induced virus. The genomic sequence of the induced virus is phylogenetically related to the evolutionarily young endogenous CrERVs described so far. We also describe the level of replication block of CrERV on deer cells and its capacity to establish superinfection interference.

Introduction

Endogenous retrovirus sequences constitute an integral part of all vertebrate genomes. They are generated following infection of the germline lineage of the host by an exogenous retrovirus and subsequent vertical inheritance of the integrated provirus form (Feschotte and Gilbert, 2012). The ERVs are classified into a large number of groups, whose diversity exceeds the currently circulating retrovirus species (Blomberg et al., 2009; Hayward et al., 2015). After the initial establishment of an integrated virus copy, which serves as a founder for a specific ERV group, further amplification and creation of new copies is enabled either by reinfection or by intracellular retrotransposition in the germline (Dewannieux et al., 2004; Jern and Coffin, 2008; Kanda et al., 2013).

Uncontrolled proliferation of ERVs in the genome would cause a burden for the host through mutagenic and various other effects. Therefore, there are multiple mechanisms that keep ERV expression and replication under control, most notably by transcriptional silencing (Liu et al., 2014; Rowe and Trono, 2011; Turelli et al., 2014). On the other hand, ERVs can be utilized for protection of the host from infecting retroviruses, a concept dubbed as “fighting fire with fire” (Malfavon-Borja and Feschotte, 2015). There are several well-documented cases in chickens, mice, cats and sheep, where endogenous envelope (Env) proteins can prevent the cell surface receptors from interacting with incoming retrovirus, resulting in a block of cellular entry (Malfavon-Borja and Feschotte, 2015). ERV-encoded proteins can also cause inhibition at several post-entry stages of infection (Arnaud et al., 2007b; Best et al., 1996; Monde et al., 2012). Another important way how ERVs can influence the outcome of retroviral infection is through recombination. ERV genomes can recombine among different endogenous loci or with related exogenous retroviruses. This can lead to the generation of fully infectious virus from two defective ERV genomes, or to the altered properties, for example altered tropism, of the exogenous partner involved in the recombination (Anai et al., 2012; Levy, 2008; Paprotka et al., 2011; Shimode et al., 2015; Young et al., 2012). In addition, through recombination with cellular genes, ERVs can form acutely transforming retroviruses (Kozak, 2015).

A practical classification is to consider ERVs as either “ancient” or “modern”, based on the time when they infiltrated the host genome (Armezzani et al., 2014). Most ERVs belong to the ancient category, where the genome invasion occurred long time ago in the evolutionary history of the host species, usually before the last speciation. Consequently, the individual ERV integrations are fixed in the host population, or even shared in phylogenetically related species. Modern ERVs entered the host genome more recently, mostly after speciation. Such ERV integrations have typically not yet reached fixation or been lost from the host lineage. At that stage, they are present in some individuals and absent in others,

* Corresponding author.
E-mail address: daniel.elleder@img.cas.cz (D. Elleder).
[1] Authors contributed equally.

http://dx.doi.org/10.1016/j.virol.2015.07.003
0042-6822/

which is denoted as insertional polymorphism. There are only a few well-studied examples of modern ERVs, these include the koala retrovirus (KoRV), endogenous Jaagsiekte sheep retroviruses (enJSRVs), porcine endogenous retroviruses (PERVs), endogenous feline leukemia viruses (enFeLVs) and other feline endogenous retroviruses (ERV-DCs), various mouse ERVs, and cervid endogenous gammaretrovirus (CrERV). The research on these viruses has lead to important insights into the process of genome invasion by an ERV and of the changes that accompany endogenization (Anai et al., 2012; Arnaud et al., 2007a; Lavillette and Kabat, 2004; Li et al., 2012; Oliveira et al., 2007; Tarlinton et al., 2006). In koalas, sheep and cats and mice, exogenous counterparts of the respective ERVs are circulating in natural populations and are associated with disease (Armezzani et al., 2014; Kozak, 2015; Levy, 2008; Xu et al., 2015). Replication-competent variants of PERV have also been reported (Preuss et al., 2006). In our previous studies we have advanced knowledge of the CrERV-mule deer model (Bao et al., 2014; Elleder et al., 2012; Kamath et al., 2014; Wittekindt et al., 2010). We have described an extensive collection of thousands of polymorphic endogenous retrovirus integration sites, comprehensively documenting the recent invasion of mule deer genomes by CrERV. The integration site patterns in individual deer were analyzed and revealed fine population structure and history of wild mule deer populations, with better resolution than in a parallel analysis performed with microsatellite markers (Kamath et al., 2014). However, all our previous work was focused on analysis of the integrated CrERV DNA or of the viral RNA expression. We have never obtained conclusive evidence of virus production or replication. Therefore, we attempted to replicate a previously published experiment (Aaronson et al., 1976), where primary blacktail deer (*O. hemionus colubmianus*, a subspecies of mule deer) cells were cocultured with human cell line. This led to the induction of replication competent gammaretrovirus species of hitherto unknown sequence, denoted deer kidney virus (DKV) (Aaronson et al., 1976; Barbacid et al., 1980).

In this study we report a successful induction of replication-competent CrERV from coculture of deer cells with a susceptible human cell line. We have characterized the physical properties of the induced virus, its phylogenetic relatedness to known endogenous CrERV copies, and its infectivity on deer and human cells. We also analyzed the capacity of the induced virus to establish interference to superinfection.

Results

CrERV can be induced by coculture of deer and human cells

Black-tailed deer primary kidney cells (OHK) and a human rhabdomyosarcoma cell line A673 were used in the coculture experiment. These cells were the same as those used in the original protocol (Aaronson et al., 1976). After approximately 30 days, RT activity in culture medium could be detected by a sensitive product-enhanced RT (PERT) assay (Fig. 1). At this point we stopped adding the OHK cells, which had served as a source of the induced virus. Due to much faster growth, only the human cells remained presumably in the subsequent continuation of the coculture. The RT activity continued to increase and eventually reached a plateau. The resulting RT level was still very low, approximately a thousand times lower than the values obtained for another endogenous gammaretrovirus, PERV (porcine endogenous retrovirus). The human and deer cells cultured separately tested negative in the PERT assay (data not shown). To confirm the identity of the induced virus species, viral cDNA was prepared from ultracentrifugation-concentrated culture fluids. Sequences highly identical to CrERV were obtained (full sequence of induced CrERV [CrERV-IND] is reported below). Therefore it is highly probable that DKV described by Aaronson, from which no sequence data is available, is identical to our recently reported CrERV.

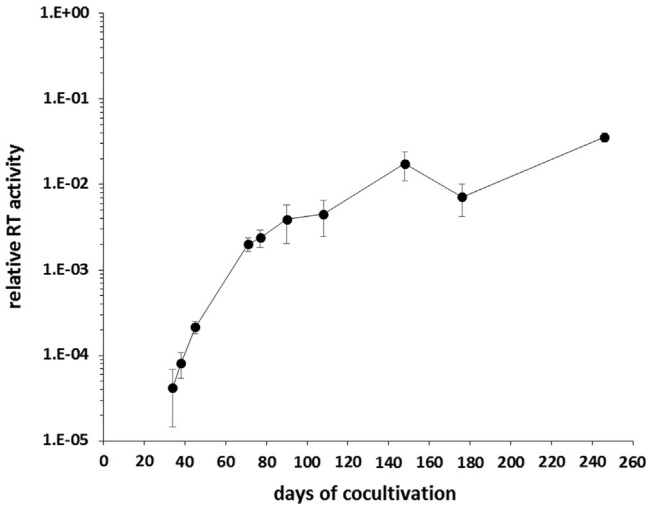

Fig. 1. Induction of virus from *O. hemionus* primary kidney cells cocultivated with human rhabdomyosarcoma cell line. At the indicated times, the RT activity in the culture supernatants was measured by a sensitive PERT assay. The results are expressed as means and standard deviations from triplicate assays.

CrERV particles

Next we examined whether the RT activity obtained from the coculture experiment belonged to particles of expected retrovirus buoyant density. The pelleted CrERV-IND was separated on iodixanol gradient and individual gradient fractions were tested by the PERT assay. For comparison we used virus particles of well-described endogenous gammaretrovirus, PERV (Bartosch et al., 2004). The RT activity peaked around the expected density of 1.1 g/ml, typical for retrovirus particles (Contreras-Galindo et al., 2012) (Fig 2A). We also obtained electron micrographs of both CrERV-IND and PERV (Fig. 2B).

The induced CrERV is infectious and xenotropic

Then, we evaluated the infectivity of the CrERV-IND particles. The virus inoculum from the coculture was used to infect naïve human and deer cells, and infectivity was assessed by the appearance of RT activity in the culture medium. Both human A673 and HEK 293 T cells could be infected (Fig. 4), however no RT activity was detected upon infection of deer OHK cells (data not shown). This is consistent with xenotropic characteristics of the induced virus, and is in agreement with observations reported by Aaronson et al. (1976) for DKV.

Relationship of induced CrERV sequence with known endogenous proviruses and construction of infectious molecular clone

To obtain the full sequence of CrERV-IND, we have amplified the provirus DNA from CrERV-infected HEK 293 T cells by long-range PCR. This sequence (deposited in Genbank under accession number KP261824) was 9,027 nucleotides long and had high identity across the entire length with a previously reported complete provirus genome, denoted CrERV-in7 (Elleder et al., 2012). There were intact open reading frames for all viral genes, *gag*, *pro/pol*, and *env* (Fig. 3B). We performed phylogenetic comparison of CrERV-IND with a set of previously published twelve endogenous CrERVs (Kamath et al., 2014). Because the full sequences of these CrERVs are not known, we have used an alignment of approximately 1.1 kb region in the 3'end of the virus genome to create the phylogeny. This region was identified