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Human cytomegalovirus (HCMV) nuclease: implications for new strategies in gene therapy

Minireview

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I. Introduction

Human cytomegalovirus (HCMV), one of eight human herpesviruses, can cause serious illness in neonates as well as in immunocompromised adults (Alford and Britt, 1993). Transplant and AIDS patients, e.g. may develop life-threatening diseases as a consequence of primary infection or reactivation of latent infection. Additionally, HCMV infections are also associated with congenital neurological complications in approximately 7,500 newborns annually (Alford and Britt, 1993). The current drugs are toxic and cause additional complications including drug resistance. Since present therapeutical approaches are limited new strategies are needed that may result from a better understanding of viral molecular biology.

The initial step of production of new virions is the packaging of newly synthesized, concatenated viral DNA into procapsids. As a consequence, blocking of this step will prevent production of viral progeny. Recently a new highly conserved gene product of ORF UL56, p130 (pUL56), was identified and partially characterized (Bogner et al., 1993). The homologous proteins of herpes simplex virus 1 (HSV-1), ICP 18.5 (UL28), and of pseudorabies virus (PrV) were reported to play an important role in DNA packaging. Viral mutants failed to cleave concatenated viral DNA which leads to an accumulation of naked nucleocapsid and uncleaved concatenated DNA in the nucleus (Addison et al., 1990; Mettenleiter et al., 1993). These reports suggested that UL56 may also play a role in virus assembly. The mechanism of DNA translocation into the procapsid and that of end formation by concatemer cutting at packaging sites (pac) are not well understood. Recently it was shown that HCMV p130 has the ability to interact with specific HCMV DNA packaging motifs and to cleave DNA bearing this motifs. Possible implications of this finding, its relation to the function of another HCMV protein, pUL89, the so-called terminase, and to the bacteriophage system are discussed in this review.

II. HCMV p130 (pUL56) is a sequence specific nuclease

Viral DNA-replication results in the formation of large head-to-tail DNA concatemers (Ben-Porat and Rixon, 1979), and maturation into unit-length molecules involves site-specific cleavage at sequences (pac motifs) located within the *a* sequence (Spaete and Mocarski, 1985). Unit-length DNA is encapsidated in the nucleus and the DNA-containing C-capsids bud into the cytoplasm through the nuclear membranes. The final event is the envelopment in the TGN and the release into the extracellular space.

The process where newly synthesized viral DNA is cleaved and packaged into preformed capsids has long been of interest, because this is the initial step in viral assembly. The HCMV genomic *a* sequence is a short sequence located at both termini of the genome and in inverted orientation at the L-S junction (Mocarski et al., 1987; Tamashiro and Spector, 1986). The *a* sequence plays a key role in replication as a *cis*-acting signal for cleavage and packaging (Chou and Roizman, 1985). There is evidence that cleavage and packaging of DNA are linked processes (Ladin et al., 1980). The HCMV *a* sequence contains two conserved motifs, pac 1 and pac 2. These packaging motifs have an AT-rich core flanked by a GC-rich sequence. In the case of HCMV, pac sites are located on one side of the cleavage site, whereas in other herpesvirus genomes the cleavage site is between these motifs (Marks and Spector, 1988). We sugggest that during the initial step of viral packaging, the capsid-associated protein p130 may bind to the pac sequences and promote cleavage of the concatemer.

Electrophoretic mobility shift assays with DNA probes

spanning the region of the *cis*-acting pac elements demonstrated that recombinant baculovirus-infected HCMV p130 formed specific DNA-protein complexes. These data suggested that p130, as a putative cleavage and packaging protein, attaches to the pac sequence. Furthermore it is proposed that viral DNA is taken up into the capsid being scanned along the complex from the bound *a* sequence across the L- and S-component until an *a* sequence in an identical orientation is found. The final step requires nicking of both strands at signals on opposite sites of the sequence.

Interestingly, by using circular plasmid DNA bearing a single *a* sequence as a substrate, purified baculovirus expressed HCMV p130 has an enzymatic activity that converts supercoiled plasmid DNA into open circular as well as linear molecules (Bogner et al., 1998; **Fig.1**). This observation is comparable with the notion that HCMV p130 may be involved in cleavage of viral DNA. By using Apyrase, evidence was provided that in contrast to the adeno-associated virus origin-binding protein Rep68 (Im and Muzyczka, 1990) the reaction is independent of ATP. Interestingly, baculovirus-UL28 cell extracts, containing the HSV-1 homolog ICP18.5 (pUL28), also cleaved supercoiled plasmid DNA molecules bearing the *a* sequence (**Fig.1**). It was then suggested that p130 and its herpesviral homologs are involved in cleaving of concatenated viral DNA and packaging into procapsids. It is currently under investigation, whether p130 operates with another viral protein, UL89.

III. Comparison with the bacteriophage system

Herpesviruses share common features with respect to DNA maturation with dsDNA bacteriophages. DNA replication results in high molecular weight concatenated DNA and procapsids are assembled around a protein scaffold (Black, 1989; Murialdo and Becker, 1978). Translocation of the DNA to the procapsid bacteriophages is an ATP-dependent process carried out by terminases (Feiss and Becker, 1983). Terminases are ATP-binding proteins which also bind and cleave concatenated DNA at cohesive (cos; e.g. phage) or packaging (pac; e.g. phage P22) sites (Gold et al., 1983). In the case of bacteriophage T4, procapsids are apparently filled without sequence specificic cleavage by a "headful mechanism" (Kalinski and Black, 1986; Streisinger et al., 1967). The HCMV UL89 gene product has some homology to the phage T4 gp17 terminase subunit. T4 terminase has two subunits. The large subunit, gp17, contains the ATP binding sites, the small subunit, gp16, is required for packaging of concatenated DNA (Rao and Black, 1988). There is preliminary evidence that baculovirus-UL89 cell extracts exhibit endonuclease activity (**Fig.2**). HCMV UL89 is also able to nonspecifically cleave DNA in a manner reminiscent of phage T4 terminase (Black, 1986). Terminase can use either concatemeric or monomeric DNA of any sequence and prefers nonspecific ends in monomeric DNA over pac-containing concatemers (Serwer; 1986).

Recently, Krosky et al. (1998) and Underwood et al. (1998) reported on a new drug, which is a derivative of benzimidazole ribonucleosides. HCMV mutants were selected by treatment of infected cultures with increasing amount of the drug. The mutations were mapped to the UL56 gene (Krosky et al., 1998) and to the gene product of pUL89 (Underwood et al., 1998). Based on the identical phenotypes of the drug resistant mutants, it is speculated that these viral proteins form the putative HCMV terminase.

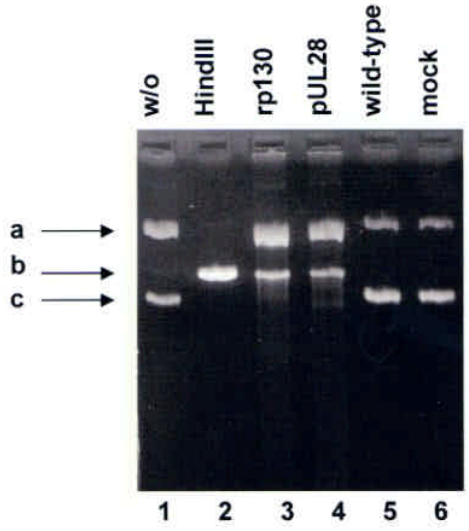

Fig.1: Nuclease activity of the p130 homolog of HSV ICP18.5 (pUL28). Nuclease reactions were incubated for 1 h at 37°C and samples were treated with proteinase K for an additional hour at 37°C. Lane 1, plasmid pON205 in the absence of protein; 2, pON205 treated with the restriction enzyme HindIII; 3, pON205 incubated with rp130; 4, pON205 incubated with HSV UL28; 5, pON205 plus wild-type infected extracts; 6, pON205 incubated with mock-infected extracts. The arrows indicate three different plasmid DNA forms: open circular molecules (a); linear forms (b); and supercoiled molecules (c).

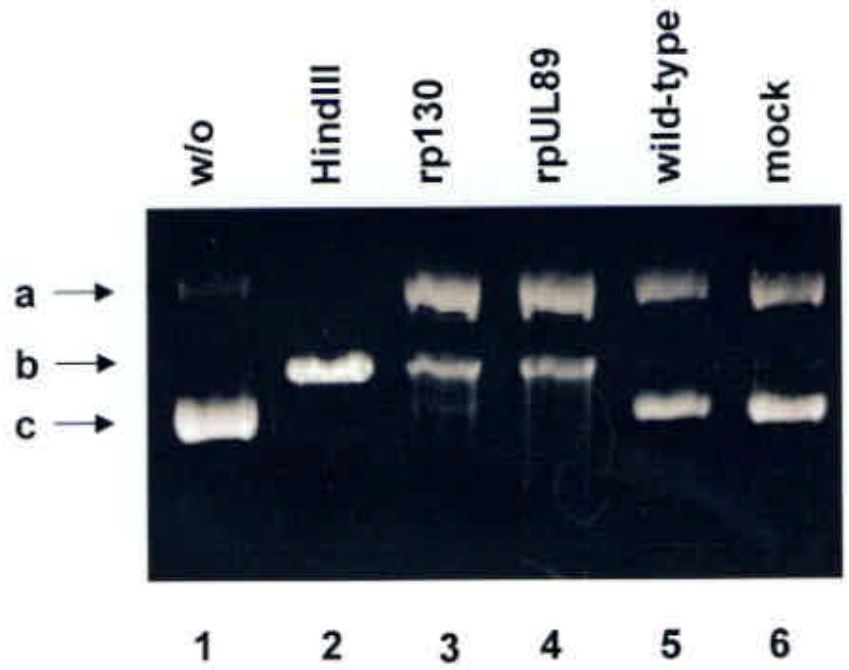

Fig.2: Nuclease activity of the T4 terminase homolog HCMV pUL89. Lanes: 1, pON205 alone; 2, pON205 treated with HindIII; 3, pON205 incubated with extract containing p130; 4, pON205 incubated with extracts containing HCMV UL89; 5, pON205 incubated with wild-type infected extracts; 6, pON205 incubated with mock-infected extracts. Open circular DNA molecules (a), linear (b) and supercoiled molecules (c) are indicated.

IV. Questions for the future

Considering that the current drugs (ganciclovir, cidofovir and foscarnet) have limited effects and dose-dependent toxicity, new antiviral therapeutics are needed. The mechanism of the current drugs is the inhibition of viral replication through an interaction with viral DNA polymerase (Erriksson et al., 1982; Ho et al., 1992; Mar et al., 1985). The inhibition of the cleavage and packaging of the viral DNA by a nuclease inhibitor may offer a potentially alternative therapy.

Taken together, the reports by Addison et al. (1990), Tengelsen et al (1993) and Mettenleiter et al. (1993), demonstrating that the HSV-1 ICP18.5 (pUL28) gene product and the PRV homolog are necessary for cleavage and packaging of concatenated viral DNA, and the observation that HCMV p130 (pUL56) can interact with specific DNA packaging motifs and is able to cleave DNA bearing these motifs, provide a basis for understanding the herpesvirus DNA packaging process at the molecular level. Identification of the structure of the proteins involved is needed as a prerequisite for the development of new antivirals. Knowledge of the three dimensional protein structure is pertinent in revealing the catalytic domain for the enzymatic activity prior to anti-viral drug-design. Regarding that mammalian cell DNA replication does not involve cleavage of concatemeric DNA, drugs targeted to the viral nucleases should be safe and selective. Therefore, our findings may help to develop new nontoxic anti-HCMV reagents for treatment of the immunocompromised patient population.

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