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Review article

Molecular Miology of the Poliovirus

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Abstract

The poliovirus is a small, and non-enveloped virus. The RNA genome of poliovirus is continuous, linear, and has a single open reading frame. This polyprotein precursor is cleaved proteolytically to yield mature products. Most of the cleavages occur by viral protease. The mature proteins derived from the P1 polyprotein precursor are the structural components of the viral capsid. The initial cleavage by 2A protease separates the P1 structural protein precursor from the P2-P3 non-structural precursor. The 2A protease is indirectly involved in the cleavage of a cellular protein p220, a subunit of the eukaryotic translation initiation factor 4F. This cleavage leads to the shut-off of cap-dependent host cell translation, and allows poliovirus to utilize the host cell machinery exclusively for translation its own RNA, which is initiated by internal ribosome entry via a cap-independent mechanism. The functional role of the 2B, 2C and 2BC proteins are not much known. 2B, 2C, 2BC and 3CD proteins are involved in the replication complex of virus induced vesicles. All newly synthesized viral RNAs are linked with VPg. VPg is a 22 amino acid polypeptide which is derived from 3AB. The 3C and 3CD are protease and process most of the cleavage sites of the polyprotein precursor. The 3C protein is also involved in inhibition of RNA polymerase II and III mediated transcription by converting host transcription factor to an inactive form. The 3D is the RNA dependent RNA polymerase. It is known that poliovirus replication follows the general pattern of positive strand RNA virus. Plus strand RNA is transcribed into complementary minus strand RNA that, in turn, is transcribed for the synthesis of plus strand RNA strands. Poliovirus RNA synthesis occurs in a membranous environment but how the template RNA and proteins required for RNA replication assemble in the membrane is not much known. The virus particle formation takes place near the RNA replication complex of the smooth membrane fraction. The RNA requirements for the encapsidation of the poliovirus genome (packaging signal) are totally unknown. The poliovirus infection cycle lasts approximately 6 hours.

Key words : *polyprotein precursor, p220, vivus induced vesicles, VPg*

Introduction

Poliovirus is taxonomically classified as a member of the family Picornaviridae. The family Picornaviridae consists of four subfamilial generas. Poliovirus is included in the subfamilial genus Enterovirus[44]. Poliovirus is serologically divided into 3 different serotypes(type 1, 2, 3). Nucleotide sequence homology among 3 serotypes is 71%. The homology in the amino acid sequences among the 3 serotypes is 88%[44]. Poliovirus has been intensively studied over the last 50 years. The entire nucleotide sequence of the genome was determined[43]. The generation of a complete cDNA clone and the application of recombinant DNA technology to the study of poliovirus has facilitated the understanding of the molecular biology of the virus[66]. The purpose of this review

is to summarize the recent progress on the molecular biology of poliovirus, and to provide insights for the utilization of the virus as pharmaceutical agent.

Virion structure and Genome of the poliovirus

The poliovirus is small, about 27 nm in diameter, and naked(non-enveloped). It consists of 60 copies of each of the capsid proteins(VP1, VP2, VP3, and VP4), and one copy of a single stranded poliovirus RNA. The RNA genome of poliovirus is continuous, linear, and contains approximately 7,500 nucleotides. The isolated RNA has a sedimentation coefficient of 35S and a molecular mass of 2.4×10^6 Da[44]. The 5' end of the viral genome is covalently linked to a small viral protein, VPg[82]. The 3' end has a run of poly(A) 40-100 nucleotides long which is genetically encoded.

Coding Region and Proteolytic Processing

A single open reading frame consisting of 6,618 nucleotides has a coding potential of 2,206 amino acids. The translation of the polyprotein initiates at the ninth AUG from the 5' end. The primary translation product would have a molecular mass of about 247 kDa. This polyprotein precursor is cleaved proteolytically to yield structural and nonstructural mature products(Fig. 1). Most cleavages of the polyprotein occur between glutamine-glycine pairs. Two cleavages occur between tyrosine-glycine pairs, and one cleavage occurs between asparagine-serine pair. The initial cleavage by protease 2A separates the P1 structural protein precursor from the nascent P2-P3 non-structural precursor. This cleavage of a tyrosine-glycine pair occurs immediately after protease 2A has been synthesized, probably by an intramolecular mechanism[79]. Most cleavages in the P2 and P3 region of the poliovirus polyprotein are catalyzed by the $3C^{pro}$. The P1 poliovirus capsid protein precursor is processed by a polyprotein consisting of a fusion between $3C^{pro}$ and $3D^{pol}$, designated as $3CD^{86}$. A final processing step is cleavage of VP0 to VP4 and VP2 at an asparagine-serine pair. This maturation cleavage occurs only after capsid assembly and is associated with encapsidation of viral RNA. It also results in structural rearrangements of the capsid and increases its stability[25]. The mechanism of this cleavage is unknown, although it has been proposed that an autocatalytic cleavage between RNA and protein might be used for this[31].

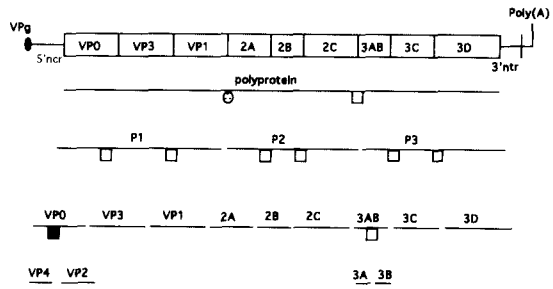

Fig. 1. Genome organization and proteolytic processing of poliovirus. VPg protein is attached at the 5' end of the virion RNA. Amino terminis of VP4 and P1 are myristylated.
□ ; glutamine-glycine cleavage by 3C or 3CD
○ ; tyrosine-glycine cleavage by 2A
■ ; maturation cleavage 5'ncr ; 5' non-coding region 3'ntr ; 3' non-translated region.

1) The P1 proteins : VP0, VP4, VP2, VP3 and VP1

The mature proteins derived from the P1 polyprotein precursor are the structural components of the viral capsid. The poliovirus capsid is composed of 60 equivalent subunits of four major intimately associated polypeptides arranged in an icosahedral lattice[36]. Capsid proteins are involved in the various capsid specific functions, e.g., protection of viral RNA, interaction with the receptor and determining antigenicity, etc.[72]. P1 polyprotein precursor and its cleaved product, the VP4 protein of poliovirus, are N-myristylated. This myristylation is required for efficient assembly of the infectious virion [7,48,58,60]. Studies from poliovirus defective interfering

particles (have deletion in P1 region) and recombinant chimeric viruses(replaced capsid region by foreign gene) showed that capsid proteins can be provided by helper virus *in trans*[18,21,65].

2) The P2 proteins : 2A, 2B and 2C

The 2A protease is a small protein with a molecular mass of 16,400 Da. It was classified as a cystein class protease because of inhibition by alkylating agents[45], but it has some structural resemblance to the two subclasses of trypsin-like serine proteases[33]. The cleavage of p220 correlates with the rapid inhibition of host cell protein synthesis that occurs after poliovirus infection. The 2A protease is indirectly involved in the cleavage of a cellular protein p220, which is a subunit of the eukaryotic translation initiation factor 4F, by activating a cellular protease which cleaves p220[47]. Another eukaryotic translation initiation factor 3, is also required for 2A protease-induced cleavage of the p220 components of eukaryotic initiation factor 4F[84]. This cleavage prevents the binding of capped mRNAs to the ribosomal 40S preinitiation complex and leads to the shutoff of cap-dependent host cell translation. This allows poliovirus to utilize the host cell machinery exclusively for translating its own RNA, which is initiated by internal ribosome entry via a cap-independent mechanism[61,62].

The functional roles of the 2B, 2C and 2BC proteins are not much known. Recent evidences showed that 2 BC and 2C proteins are involved in the intracellular formation of virus induced vesicles[1,12,17], and 2B promote the dissembly of the Golgi complex[73]. These virus induced vesicles have been found to be multiorganelle origin, including the rough endoplasmic reticulum as well as Golgi complex[75]. These virus-induced vesicles were found to form rosettes which surround the replication complex and can be isolated in a functional state. The initiation of plus-strand RNA synthesis as well as elongation of viral RNA can take place not only in rosettes but also on single vesicles[24]. Helicases are capable of enzymatically unwinding duplex RNA structures by disrupting the hydrogen bonds that maintain the two strands together. All helicase described to date also have NTPase activities[42]. The poliovirus 2C protein exhibits NTPase activity[54,69], but a helicase activity could not be demonstrated[69]. Recent report has shown that protein 2BC enhances cytoplasmic calcium concentrations. Regions present in both 2B and 2C are necessary to augment cellular free calcium level[2]. Therefore, in addition to inducing proliferation of membranous vesicles, poliovirus protein 2BC alters cellular calcium homeostasis.

3) The P3 proteins : VPg, 3AB, 3C, 3CD and 3D

The proteins of the P3 region are important for the replication of viral RNA. All newly synthesized viral RNAs are linked with VPg[82]. VPg is a 22 amino acid polypeptide which is derived from 3AB, the VPg precursor protein. The third amino acid from N-terminus of VPg is tyrosine, which forms a bridge to the 5' terminal phosphate of the genomic RNA. The predominant VPg-containing precursor polypeptide, 3AB, has a molecular mass 12,000 Da and appears to be a membrane associated protein that might have an important role in the initiation of poliovirus RNA replication[22,29,57,77].

The 3C and 3CD are protease and process most of the cleavage sites of the polyprotein precursor[44]. Poliovirus infection leads to inhibition of all three classes of host cell RNA synthesis. The 3C protease is involved in inhibition of RNA polymerase III mediated transcription by converting an active form eukaryotic transcription factor IIIC to an inactive form[19]. The TATA-binding protein, a component transcription factor IID, is cleaved directly *in vivo* and *in vitro* by 3C proteinase, leading to an inhibition of RNA polymerase II-mediated transcription[20]. Inhibition of RNA polymerase I-mediated rRNA synthesis in poliovirus-infected cells is presumably due to a decrease in a specific activity that is needed for transcription initiation[71].

The $3D^{pol}$ is the RNA dependent RNA polymerase[23,26,

[81], which is required for replication of the RNA genome of poliovirus(see RNA replication section, below).

Non-coding region sequences

1) 5'non-coding region(5' ncr)

Poliovirus RNA has unusually long 5' non-coding sequences, consisting of 740 nucleotides. A possible cloverleaf-like structure formed by the 5'-proximal end of the RNA (approximately 90 nt) is probably a cis element that regulates the synthesis of the plus-strand RNA[5]. Polioviral mRNA is identical in sequence to the genomic RNA except that the 5' terminal VPg has been removed. The 5' terminus of polioviral mRNA is not capped, but terminates in pUp. The cap structure, 7-methyl G, of cellular mRNA is used as a binding site for the cap binding complex[46]. One of the features of poliovirus infection is the inhibition of cap dependent cellular translation. Therefore, poliovirus must have a mechanism to circumvent this condition. Indeed, the sequences between 140 and 630 of poliovirus 5' non-coding region, known as an internal ribosome entry site (IRES), mediate cap-independent, internal initiation of translation[61,62]. This region of the RNA is considered to have a complicated highly ordered structure formed by a number of secondary structures. A number of host cellular factors are required for the expression of poliovirus IRES function[13,38]. Recent evidence showed that IRES could potentially be a host range determinant for poliovirus infection[76]. The RNA segment (in the region from nt 343 to 500) within the IRES also controls viral RNA synthesis[13]. This observation indicates that the regulatory elements for RNA synthesis and protein synthesis may overlap in part in the 5' ncr. Many other picornaviruses, such human rhinovirus, encephalomyocarditis virus, foot-and-mouth disease virus, coxakievirus, Theiler's murine encephalomyelitis virus, and hepatitis A virus, have been shown to utilize the IRES[9,14,27,30,38,40,41,51]. In addition to picornaviruses, many other viruses (such as hepatis C virus, murine leukemia virus, infectious bronchitis virus, pestivirus and plant potyviruses) utilize internal initiation of translation for synthesis of one or more gene products[10,27,49,50,64,68,80]. Cellular mRNAs encoding immunoglobulin heavy chain binding protein, Drosophila antennapedia protein, and the mouse androgen receptor also uses IRES-mediated initiation of protein synthesis[34,52,59].

2) 3' end non-translated region(3' ntr)

The poly(A) on the poliovirus RNA is encoded by viral genome(40-100 nucleotide residues). Poliovirus polyprotein synthesis is terminated at nucleotide 7361, 73 nucleotides preceding the poly(A) tract. Comparison of the sequences from different picornaviruses showed the sequence near the poly(A) tract have a high degree of homology. A mutant containing an 8 base insertion in the 3'ntr showed replication-deficient temperature sensitivity[11,74]. 3'ntr of all enteroviruses appears to fold into the same core structure consisting of two stem-loop domains, X and Y[37,63]. Covariance between enterovirus sequences, maintaining domains X and Y, is strong evidence that these may play an important role in viral RNA replication[63]. In addition, another typical pseudoknot has been proposed to occur in the 3' ntr of poliovirus[39].

Overview of the life cycle of poliovirus

To initiate infection, as shown in Fig. 2, virions attach to the poliovirus receptor[53] in a phase termed "attachment." After attachment, the viral capsids are rapidly altered by loss of VP4. These altered particles are internalized into the host cell via receptor-mediated endocytosis and then rapidly uncoat their RNA[44]. Since the virion does not carry the replicase, translation of replicase must take place for RNA replication. Virion RNA directs the synthesis of viral protein. Translation of the viral genome is associated with membrane bound polysomes

on the rough endoplasmic reticulum[78]. Within the first 2 hours after poliovirus infection, the synthesis of host cell proteins, RNA, and DNA are drastically reduced, a phenomenon called "shut-off." The parental plus strand RNA serves as a template for the synthesis of minus strand RNA that, in turn, will direct the synthesis of more plus strand RNA. All newly synthesized RNAs have VPg at the 5' end[82]. This newly synthesized plus strand RNA will be used in the infected cells : 1) as mRNA to direct the synthesis of viral proteins (for this to occur VPg might be cleaved by a host cellular enzyme from the genome[3], and 2) as a template to direct the synthesis of minus strand RNA(see RNA replication section, below). These early events must involve delicate regulatory mechanisms. Cellular membranes play a central role in poliovirus replication[12,24,55,75,77]. The replication complex, containing replicative intermediate RNA, the viral replicase, host proteins, and other P2 proteins as well as 3CD, is tightly associated with the membranes and capsid proteins[4,5,29,32,83].

During late infection, newly synthesized plus strand RNA will be encapsidated as virion RNA into new virions. Only plus strand RNAs containing VPg are encapsidated (see encapsidation section). Virion RNA synthesis and capsid assembly are intimately associated with the smooth endoplasmic reticulum of newly formed virus specific vesicles[15]. Previous reports suggested that RNA synthesis and encapsidation of virion RNA are tightly linked processes[15,85]. Cleavage of the capsid protein VP0 induces a final change in the virion conformation and completes maturation of the progeny virions[31]. During the last stages of the life cycle, viral particles accumulate in the cytoplasm of the cell until the progeny viruses are released from the infected cell. Very little is known about the mode of exit of the mature poliovirus particle from the host cell. Usually, the poliovirus infection cycle lasts approximately 6 hours in infected cells.

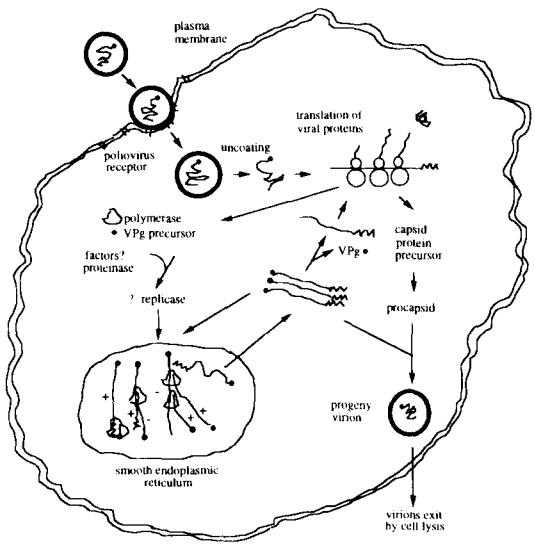

Fig. 2. Life cycle of poliovirus(modified from Kitamura et al., 1980).

RNA replication

It is known that poliovirus RNA replication follows the general pattern of positive strand RNA viruses : plus strand RNA is transcribed into complementary minus strand RNA that, in turn, is transcribed for the synthesis of plus strand RNA strands. Poliovirus RNA synthesis occurs in a membranous environment *in vivo*, but how the template RNA and proteins required for RNA replication assemble in this membrane is unknown.

A template dependent poliovirus specific RNA polymerase, now known as $3D^{pol}$, was isolated[26] and the *in vitro* properties characterized. Further studies developed an *in vitro* system to study the first step in the replication in which the plus strand RNA is copied to a minus strand[23]. In addition to the viral $3D^{pol}$, the system requires a host cell protein, host factor(HF). HF has been purified by several groups, and has been reported to have several different enzyme activities, including protein kinase[56], terminal uridylyltransferase[6], or endonuclease[35]. Recent studies have suggested that the synthesis of minus to plus strand might utilize a different mechanism, probably using protein priming with VPg[29].

Encapsidation

Early studies suggested that poliovirus capsid proteins were synthesized on polysomes of rough endoplasmic reticulum[8,70,78]. The virus particle formation takes place near the RNA replication complex of the smooth membrane fraction[15,16]. Therefore, the capsid protein must be transported from polysomes to smooth membrane, although the exact details remain unknown. In order for the RNA genome to be encapsidated, a specific RNA-capsid interaction probably needs to takes place. Two models have been proposed for the process. In the first, viral RNA is synthesized and condensed. Encapsidation occurs around the condensed RNA core[28]. In the second, the viral RNA during synthesis is directly inserted into a procapsid that is attached to the membrane[85]. The RNA requirements for the encapsidation of the poliovirus genome are totally unknown. From the fact that all the encapsidated RNA had a genome-linked protein, VPg, while mRNA did not, it was deduced that VPg might play a role in some kind of interaction between poliovirus RNA and capsid proteins[82]. Indeed, some mutants in VPg resulted in RNA replication but did not produce infectious virus[67].

Concluding Remarks

Poliovirus was the first animal virus to be purified, and obtained in crystalline form, and poliomyelitis was among the first human or animal disease found to be transmissible by pure RNA molecule. The poliovirus life cycle consists of a complex series of events which links the translation, replication and encapsidation of the viral RNA genome. Although safe and effective poliovirus vaccine has been developed already, the availability of infectious cDNA clone of poliovirus genome has made possible to define the requirements for translation, replication, and encapsidation of the viral genome[66]. The determination of three dimensional structure of the poliovirus virion by high resolution X-ray crystallography has provided insight into the understanding of many fundamental properties including neutralization of the virus[36]. In addition, identification of the poliovirus receptor[53] has important implications for understanding the virus entry mechanism of the permissive host cell. In the future, elucidation of molecular mechanism of poliovirus life cycle may provide us good opportunity to understand the interactions between poliovirus-host cells.

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초록 : 폴리오바이러스의 분자생물학

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폴리오바이러스는 바이러스들 중에서도 특히 커기가 작은 바이러스로서 피막(coat)을 둘러싸는 막(envelope) 이 없다. 폴리오바이러스는 (+) 가닥의 단일 RNA 게놈을 갖는데 이는 단지 한개의 해독판(open reading frame)을 이용하여 다단백질전구체를 만든 후 바이러스 자체의 단백질분해효소에 의해 스스로 잘라져서 궁극적으로는 특이한 기능을 갖는 여러개의 단백질이 된다. P1 다단백질전구체로부터 만들어지는 단백질들은 바이러스의 피막을 구성하는 성분이다. 단백질분해효소인 2A에 의한 최초의 절단은 구조단백질 P1 전구체와 구조단백질이 아닌 P2-P3간을 분리시켜준다. 단백질분해효소 2A는 진핵세포 판독개시인자(translation initiation factor) 4F의 한 subunit인 숙주단백질 p220의 절단에 간접적으로 참여한다. 이 단백질의 절단은 캡(cap)에 의존하는 숙주세포의 대부분의 판독을 차단하게 되며 이는 판독에 사용되는 숙주세포의 모든 기구들을 캡에 의존하지 않는 폴리오바이러스 RNA 특유의 판독을 위해 전적으로 사용할 수 있게 해준다. 2B, 2C, 2BC 단백질의 기능에 대해서는 많이 알려져 있지않다. 2B, 2C, 2BC와 3CD 단백질들은 바이러스로 인해 만들어지는 소낭(vesicle)의 복제복합체에 함유되어 있으므로 바이러스의 RNA 복제시 중요한 역할을 함을 암시해준다. 새로이 만들어진 모든 바이러스 RNA는 VPg와 공유결합으로 연결되어 있다. VPg는 3AB로 부터 만들어진 아미노산 22개 짜리의 폴리펩타이드이다. 3C와 3CD는 단백질분해효소로 다단백질 전구체의 대부분의 절단부위를 잘라준다. 3C단백질은 숙주의 전사인자를 불활성화 시킴으로써 RNA polymerase II와 III에 의한 전사를 저해한다. 3D는 RNA의존성RNA중합효소 이다. 폴리오바이러스는 (+)가닥 RNA 바이러스의 일반적인 복제양식을 따른다. 즉 (+)가닥 RNA는 이와 상보적인 (−)가닥 RNA로 전사되고 이는 다시 (+)가닥 RNA의 합성을 위한 주형으로 사용된다. 폴리오바이러스의 RNA 합성은 세포내막에서 일어나는 데 RNA 복제에 요구되는 주형 RNA와 이때 필요한 단백질들이 어떤 방법으로 세포내막에 모일 수 있는지는 아직 밝혀진 것이 적다. 바이러스입자의 형성은 세포막의 RNA복제가 일어나는 근처에서 일어나며 이때 VPg가 결합된 (+)가닥 RNA만이 바이러스 피막내로 들어가는 데 피막단백질이 (+)가닥 RNA를 인식하는 표지 즉 packaging signal에 대해서는 거의 알려져 있지않다. 폴리오바이러스 감염 후 첫 바이러스입자가 만들어지기 까지는 약 6시간이 소요된다.