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Potyviral Genome-Linked Protein and its Interaction with Plant Defense Ribosome Inactivating Protein from *Phytolacca Americana*

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Abstract

Agriculture is an indispensable part of every person's life, ensuring that nutritious and inexpensive food is readily available. Agriculture continues to be confronted with epidemics, having devastating effects on economies and the plant sources essential for human and animal life. Plants and their pathogens have developed evolutionary adaptations, each shaping the other's defence and invasive strategies. Many different plants produce toxic ribosome inactivating proteins that aid in their defence mechanisms against pathogenic invaders. Viruses must adapt to the host translational machinery, several having evolved to include viral genome-linked proteins that carry numerous viral functions. Here, we review how a *potyviral* protein from turnip mosaic virus linked to its genome is able to inhibit pokeweed plant defence protein, and perhaps potentially conferring viral resistance to the toxin.

Introduction

For eons, plants and their pathogens have been developing and shaping survival strategies. Plant defence mechanisms include preformed and induced defences, which prevent pathogens from entering the plant cell, limit availability and/or restrict nutrients necessary for the growth and replication of the pathogen [1-3]. Essential pre-invasive defences include: physical barriers that prevent access of the pathogen, leading to the inability of most microbes to infiltrate outer epidermal wall [4]. The plant actin cytoskeleton network is another important impediment encountered upon pathogen ingress [5]. Chemical barriers, including phytoanticipins, have many roles in plant development and growth; many have evolved to affect pathogenesis. Some examples include saponin glucosinalates, sterols, and glycoalkaloids [6,7].

Ribosome inactivating proteins (RIPs)

Many members of the kingdom of Planta manufacture protein phytotoxins that include various lectins, pore-forming toxins, antimicrobial proteins, protease inhibitors, arcelins, and ribosome inactivating proteins (RIPs) [8]. RIPs are believed to play a vital role in plants defence mechanisms against foreign pathogenic invaders. The toxicity of several RIPs has been explored since antiquity for their homicidal capabilities, with such well-known examples as ricin (from *R. communis*) and abrin toxin (from *A. precatorius*) [9]. RIPs are RNA N-glycosidases [10] that cleave adenines selectively from the conserved sarcin/ricin loop (SRL) of prokaryotic and eukaryotic large rRNAs, inhibiting protein synthesis [11,12]. American pokeweed plant (*Phytolacca americana*) and common soapwort *(S. officinalis*) produce pokeweed antiviral protein (PAP) [13] and saporin [14], respectively; both exert potent antifungal and antiviral properties. Commonly, RIPs being potent cellular toxins are exported out of the cell once they are synthesized, and localized within the cell wall matrix [15-17]. It is hypothesized that RIPs gain access into the cytoplasm as the pathogen enters the cell, thus promoting their antiviral activity by impairing host ribosomes [18,19].

RIPs are categorized into two major classes based on their physical properties: holo-RIPs and chimero-RIPs [20]. Holo-RIPs consist exclusively of a single RNA N-glycosidase catalytic domain. Most holo-RIPs consist of a single, intact polypeptide of approximately 30 kDa and are often referred to as type 1 RIPs [13,21,22]. Examples of holo-RIPs include PAP, saporin, and barley (*H. vulgare*) translational inhibitor. The majority of RIPs characterized thus far fall into this category [21]. Chimero-RIPs are constructed of one or more protomers consisting of catalytic N-glycosidase domain (A chain) linked through a disulfide bond to a structurally and functionally different domain with carbohydrate binding properties (B chain). Most chimero-RIPs are known as type 2 RIPs, like ricin and abrin, and are acutely toxic heterodimeric proteins, each of approximately 30 kDa [23,24]. The type 2 RIPs have been quite valuable for studies of endocytosis and intracellular transport in mammalian cells [16,25,26]. Some chimero-RIPs are rather classified into type 3 class RIPs, which are much less common. Type 3 RIPs are synthesized as inert precursors (proRIPs) that undergo proteolytic modifications, allowing for acquisition of full enzymatic activity [20]. Presently, type 3 RIPs have been identified from maize (*Z. mays*) and barley [27-30].

American pokeweed plant produces several PAP isoforms [22]. PAP-I (or simply PAP), PAP-II and PAP-III are leaf isoforms that appear in spring, early summer and late summer respectively [13,22,31-35], whereas PAP-S1 and PAP-S2 are isoforms isolated from seeds that exhibit the highest activity *in vitro* of all the isoforms [36-38]. A further isoform, α-PAP, is similar in sequence to PAP-S1, and essentially expressed in all organs of the plant [38,39]; it shares 74% identity with PAP. PAP-R has been isolated from the roots of pokeweed plant [40,41] and PAP-H is from hairy roots [40]. Interesting to note that RIP-free callus and suspension cultures of *P. americana* have been acquired [40,42]. All PAP isoforms present prominent antiviral characteristics with high anti-ribosomal activity [31], and the molecular antiviral mechanism of PAP has been deciphered [43]. Examination of PAP's viral selectivity is of pivotal importance, for it lowers infectivity of many plant and animal viruses, such as HIV-1 [44], human T-cell leukemia virus-1 (HTLV-1) [45],

herpes simplex virus (HSV) [46], brome mosaic virus (BMV) [47], tobacco mosaic virus (TMV) [42], influenza [48], hepatitis B virus (HBV) [49], and poliovirus [50]. Understanding of PAP antiviral mechanism will contribute to the development of practical solutions for the control of plant, animal and human diseases, and is important for design of novel efficient antiviral agents through genetic modifications, control of signaling mechanisms, or other therapeutic agents.

Positive strand RNA viruses: the genus *potyvirus*

The majority of positive strand plant RNA viruses differ from the typical 5′-cap/3′-poly(A) tail organization found in host mRNAs. The cap and poly(A) tail increase the stability of mRNA, and recruit translation initiation factors, supporting a format of closed loop mRNA translation [51]. The assorted collection of cis-acting motifs, found in numerous viral mRNAs, compensate for the lack of a cap, poly(A) tail, or both. Elaborate higher-order structural non-coding elements in the 5′ and 3′ untranslated regions (UTRs), or tRNA-like structures (TLS) of viral transcripts, aid in the recruitment of translation factors, leading to the preferential translation of viral genes [52-55]. Zeenko and Gallie [54] showed the 5′-UTR of tobacco etch virus (TEV) includes an internal ribosome entry site (IRES). This allows ribosomes to dock, leading to the initiation of viral RNA translation.

The genus *Potyvirus* includes over two hundred members and is classified as one of the most extensive plant virus family – *Potyviridae* [56]. The genome of *Potyviruses* is comprised of approximately 10 kb positive-sense single stranded RNA molecule, covalently connected to a viral protein (VPg) at the 5′ end via a tyrosine residue [57], and poly(A) tail at the 3′ end [58-60]. The *potyviral* RNA contains a single open reading frame, translated into a large polyprotein, proteolytically cleaved into mature proteins by specific virus-encoded proteases [61]. This viral protein is known to serve as an analog of the 5′-m7G cap of the mRNAs, and has been shown to play an important role in mRNA translation since it interacts with the cap-binding proteins (e.g., eIF4E, eIFiso4E, eIF4F, eIFiso4F) [62,63]. VPg is vital for the infectivity of the virus [64], cell-to-cell movement [65-68], and has been linked to an array of other viral functions. Khan et al. [69] have revealed that potyviral VPg stimulates the *in vitro* translation of uncapped IRES-containing RNA, while inhibiting capped RNA translation in wheat germ extract. These effects have shown to be dependent on VPg-eIF4E(4F) or VPg-eIFiso4E(iso4F) interactions. These studies demonstrate that VPg competes for the cap-binding site in these translation initiation factors. Binding studies [63] show that VPg and cap bind competitively to eIFiso4E.

Interactions between pokeweed antiviral protein and *potyviral* protein

Recent studies examined the interactions between PAP (cap-binding protein) and VPg from TuMV [70], and revealed that VPg competes with TEV RNA for PAP binding [71]. These PAP-VPg interactions are enthalpically-driven and entropically favorable [70], exhibiting a similarity to those of eIFiso4E- and eIFiso4F-VPg binding [63]. Moreover, PAP demonstrated greater affinity for this viral protein, as compared to m7GTP-cap analog [35] and eIFiso4F [63]. PAP, having greater binding affinity for VPg than that for the cap structure, would certainly create an advantage for the cell if VPg were to target PAP toward viral RNA for depurination. Interestingly, we have determined that VPg displays strong inhibitory effect on PAP's activity, decreasing the amounts of purines released from different RNAs (SRL oligo RNA, TEV RNA and luciferase mRNA) [70], implying that VPg may contribute to a viral strategy of overcoming one of the potential host cell defence mechanisms – the depurinating activity of PAP. This is further supported by Baldwin et al. [35], and solidifies the accepted function of PAP as a RIP.

Conclusion

Generally, viral RNA is translated less efficiently than capped host RNA, and has to compete for available cell resources to sustain translation. Formation of VPg-eIFiso4F complex would lead to a non-productive complex, reducing host cell protein synthesis. Conversely, VPg-eIF4F complex would also lead to the inhibition of capped mRNA translation, but in this case the complex would bind more tightly to IRES-containing mRNA leading to the preferential production of viral protein [63]. These complementary functions offer a significant competitive advantage for viral RNA translation. Plant-pathogen interactions continuously drive rapid evolutionary changes on both sides of the interactions. Plants produce toxic proteins that help them in battle viruses; meanwhile, viruses develop even more elaborate strategies to overcome these plant defence mechanisms. Here we see an example of how viral genome-linked protein may confer resistance to plant defence mechanisms. Further studies of VPg inhibitory effects on the activity of other RIPs may provide researchers with new avenues to design novel and natural protein inhibitors of RIP cytotoxicity [22,70].

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