Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Virology

Use of peptide aptamers, cationic peptides and artificial zinc finger proteins to generate resistance to plant viruses

Takashi Sera

Various RNA/DNA viruses have caused severe infectious diseases in plants as well as animals, including humans, and been a threat to the production of agricultural crops. Therefore, prevention of plant virus infections is a major objective in crop protection. One attractive approach is to inhibit functions of viral proteins responsible for virus infections. In this review, I describe the status using such approaches to confer virus resistance to plants by three types of peptides/proteins: peptide aptamers, artificial zinc finger proteins and acidic peptides. These approaches vary in their specificity, broadness to other viruses, extent of protection and mechanisms of action. Additional ways to improve these approaches are also discussed.

Address
Department of Applied Chemistry and Biotechnology, Graduate School of Natural Science and Technology, Okayama University, Tsushima-Naka, Kita-ku, Okayama 700-8530, Japan

Corresponding author: Sera, Takashi (sera@cc.okayama-u.ac.jp)

Current Opinion in Virology 2017, **26**:120–124

This review comes from a themed issue on **Engineering for viral resistance**

Edited by **John Carr** and **Peter Palukaitis**

For a complete overview see the Issue and the Editorial

Available online 12th August 2017

http://dx.doi.org/10.1016/j.coviro.2017.07.023

Introduction

Viruses cause severe infectious symptoms not only in humans and animals but also in plants, and the countermeasures have become one of important issues for crop protection. However, unlike other microorganisms, pesticides effective for viruses have not been developed, and various attempts to prevent virus infections such as RNA interference and genome editing have been made so far. This brief review introduces three types of peptides/proteins: peptide aptamers, artificial zinc finger proteins, and cationic peptides, to control plant viruses (Table 1). One of the common advantages of these approaches is that it has only a minimum of necessary domains so that it is possible to minimize unpredictable or detrimental effects such as those seen with viral proteins.

Peptide aptamers

Peptide aptamers are short peptides [20–30 amino acids (aa)] designed or selected from a random peptide library to bind to specific proteins and interfere with their functions *in vivo* [1]. Peptide aptamers have been successfully used in *E. coli*, yeast, *Drosophila*, and cultured human cells. They are used not only as a basic research tool, but also for drug target validation and drug discovery [1–6]. Peptide aptamers were also applied to plant viruses. Similar approaches include uses of antibodies such as single-chain antibodies, but the expression of antibodies in the plant cytoplasm is generally difficult [7]. Another advantage is to minimize unpredictable or potential deleterious effects observed in the expression of functional viral proteins [8,9] because peptide aptamers contain only minimum functional domains.

Peptide aptamers from a native protein

Peptide aptamer technology was first applied to tomato spotted wilt virus (TSWV) as a plant virus [10••]. The peptide aptamers were derived from the TSWV nucleoprotein. Because the homopolymerization of the nucleoprotein is required for the viral infection cycle, the inhibition of the process was aimed to prevent TSWV infection. For this purpose, a library of random fragments of the TSWV *Nucleoprotein* gene was constructed. Peptide aptamers that bind to the nucleoprotein were selected from the library by using a yeast two-hybrid system in which a TSWV nucleoprotein mutant having no homopolymerization ability was used as the bait. One advantage of this approach is to select functional peptide aptamers interacting with the bait protein in living cells unlike *in vitro* selection systems such as phage display.

Using this system, a 29-aa peptide (amino acids 220–248 of the TSWV nucleoprotein) was selected. This peptide was fused to β-glucuronidase as a carrier protein and transgenic plants expressing the fusion protein was then generated. When these transgenic plants were challenged with TSWV, many transgenic lines expressing the aptamer-β-glucuronidase fusion demonstrated complete resistance with no symptoms, while all inoculated control lines developed symptoms and eventually died. Note that symptomless infection in the transgenic lines was not specifically assessed. Interestingly, the transgenic lines

Table 1

Peptides/proteins for plant protection, their target, their application, and their mechanisms of action

	Name	Target	Application	Mechanism of action	Reference
Peptide aptamers	N/A	Nucleoprotein	TSWV	Inhibition of nucleoprotein's function	[10••]
	N/A	Rep	TGMV	Inhibition of Rep's function	[11••]
	N/A	Rep	TYLCV, ToMoV	Inhibition of Rep's function	[13••]
Artificial zinc finger proteins	N/A	Direct repeats	BSCTV	Inhibition of Rep binding	[20••]
	N/A	Direct repeats	TYLCV	Inhibition of Rep binding	[23]
Cationic peptides	Melittin analog	Coat protein	TMV	Unknown	[29••]
	10R, 11R	Unknown	TMV	Unknown	[35••]
	PV5	Unknown	TMV	Unknown	[36•]

also demonstrated resistance to other tospoviruses, such as groundnut ringspot virus and chrysanthemum stem necrosis virus, as expected from the experimental results of yeast two-hybrid analyses against these viruses. This indicates that the targeting of evolutionary conserved functions of viral proteins in planta can be used to engineer broad-spectrum virus resistance.

Peptide aptamers from a random peptide library

Peptide aptamers also have been selected from a random library [11••]. Peptide aptamers alone are very flexible and necessary to be constrained to increase affinities to a target protein [12]. Thus, a peptide aptamer domain was constrained in the active side of the thioredoxin A (TrxA) gene. The library used encoded *E. coli* TrxA with 2.9×10^9 random 20-mer peptides in its active site [1]. Peptide aptamers were selected from this library to bind to the AL1 replication-associated protein (Rep) of tomato golden mosaic virus (TGMV), using the yeast two-hybrid system. Thus, 597 positive candidates were isolated from a screen of 2×10^7 yeast colonies and 88 unique TrxA peptides were selected. Among them, some selected peptide aptamers interfered with TGMV replication in assays using protoplasts isolated from *Nicotiana tabacum* (BY-2) suspension cells. Note that TGMV resistance by these aptamers in planta was not examined in the study. This research group investigated further 16 selected peptide aptamers among those selected in their previous study [13••]. They examined whether these 16 aptamers bound to the Rep proteins derived from nine viruses representing the three major *Geminiviridae* genera by the same approach with the yeast two-hybrid system. They found that the two aptamers, A22 and A64, had the highest binding to the TGMV Rep protein and also interacted with the Rep proteins derived from the other eight and seven geminiviruses, respectively. Then, the A22 and A64 peptide aptamers were expressed transgenically from the genome of tomato (*Solanum lycopersicum* cv. Micro Tom) and the resistance of these transgenic tomato to tomato yellow leaf curl virus and tomato mottle virus was investigated. These viruses have different lineages (Old World versus New World) and genome structures (monopartite versus bipartite), among the above nine geminiviruses. They demonstrated that these transgenic lines effectively delayed viral DNA accumulation compared with wild-type tomato. Their peptide aptamers selected from a random peptide library to bind to the TGMV Rep protein also demonstrated broad-spectrum virus resistance. However, as time passed from 14 to 28 days post-infection, more viral DNA accumulated and symptoms developed further, indicating that the best peptide aptamers did not completely inhibit virus replication under their experimental conditions.

Short summary of peptide aptamers

In order to select effective peptide aptamers by a random peptide library, it is essential to use an effective selection system that can manage high-throughput selection of a large library. In the case of a limited library size, the yeast two-hybrid system seems to be very useful. This system enables the selection of peptides functional in cells, and is a simple method when a viral protein is targeted for aptamer selection because viral proteins are exogenous in yeast cells. However, the yeast system has a significant drawback, in that it can handle a limited library of 10^6–10^7 cells at most, because the library size depends on the transformation efficiencies of cells used for selection. As shown above, only 2×10^7 cells were screened from the yeast two-hybrid system even though a larger library of 2.9×10^9 random 20-mer peptides was generated in *E. coli*, indicating that 99% of random peptides were not actually applied to the yeast selection. Theoretically, yeast systems can screen only a permutated peptide library of up to a 5-mer peptide ($20^5 = 3.2 \times 10^6$).

It is likely that the phage display system is superior to the yeast system when novel peptide aptamers are required to be selected from a huge library such as a random peptide library, because the phage display system successfully selected functional single-chain antibody *in vitro* [14]. However, the phage display system can only screen a library of up to 10^{10} variants. Accordingly, to create better peptide aptamers, by utilizing the benefit of variety in a huge random peptide library, cell-free selection systems such the mRNA display [15] and ribosome display [16] would be adequate. Otherwise, a two-step selection may be more realistic and practical: first, a huge library of