

Molecular biological studies on the interactions between bromoviruses and plants

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This article is an abstract of the presentation by a winner of the Society Fellowship at the 2024 Annual Meeting of the Phytopathological Society of Japan in Sendai.

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Introduction

Viruses are one of the important pathogens in crop diseases. When viruses infect plants, interactions between viral and plant factors determine the success or failure of infection. These interactions affect virus replication, cell-to-cell movement, systemic infection, disease symptom development, and induction of host defense responses. Although the viral factors involved in each process have been rapidly analyzed in recent years, there are few reports on the host factors and their interactions with each other, which is a major barrier to the elucidation of the entire mechanism of viral infection and the development of control methods for viral diseases. We have been studying virus-plant interactions using bromoviruses as models to elucidate the mechanisms of virus infection.

Establishment of gene manipulation system for bromoviruses

Bromoviruses have a three-segmented, positive-sense single-stranded RNA genome (RNA1, RNA2, and RNA3). RNA1 and RNA2 encode 1a and 2a proteins, respectively, which are replication enzyme components. RNA3 encodes 3a movement protein (MP), which are necessary for viral cell-to-cell movement and coat protein (CP) (Bujarski 2008). CP is translated from subgenomic RNA4, which is transcribed from RNA3. In brome mosaic virus (BMV), a type species of the genus *Bromovirus*, the entire genomic RNA sequence of the Russian strain of BMV was first determined in 1984 by Dr. Paul Ahlquist's group at the University of Wisconsin, USA (Ahlquist et al. 1984a). In the same year, the same group established the first gene manipulation system for plant RNA virus in which infectious viral RNA transcripts can be synthesized in

vitro via full-length cDNA (Ahlquist et al. 1984b). The whole genome sequences of the closely related cowpea chlorotic mottle virus (CCMV; Allison et al. 1988) and broad bean mottle virus (BBMV; Romero et al. 1993) have also been determined, and genetic manipulation systems have been established. We established a genetic manipulation system in the ATCC66 strain of BMV (Mori et al. 1991), sequenced the whole genome, and performed a comparative analysis with the Russian strain (Mise et al. 1994). In the BMV-ATCC66 strain, the viral particles contain two different molecular weights of CP, and the mechanism of generation of CP is that a two-base deletion immediately upstream of the start codon of the CP gene encoded by RNA4 reduces the translation efficiency of the full-length CP, and a second, shorter CP from a second start codon seven codons downstream from the original start codon is generated (Mise et al. 1992). Three other species in the genus *Bromovirus* are known, and we have established gene manipulation systems for spring beauty latent virus (SBLV; Fujisaki et al. 2003), cassia yellow blotch virus (CYBV; Iwahashi et al. 2005), and melandrium yellow fleck virus (MYFV; Narabayashi et al. 2009) and reported the genome structure and unique features of each virus.

Analysis of bromovirus factors required for infection in plants

We have studied host specificity between viruses and plants using BMV, which primarily infects grasses such as barley, and CCMV, which primarily infects legumes such as cowpea. First, both chimeric viruses systemically infected *Nicotiana benthamiana*, a common host of both viruses, by exchanging MP genes between BMV and CCMV. These results suggest that there is no problem in the interaction between MPs derived from the two bromoviruses and other viral factors. However, each chimeric virus lost systemic infectivity in its original hosts, barley and cowpea, indicating that the MP gene is important for the host specificity exhibited by BMV and CCMV, and furthermore, the specific interaction between the MP gene and host plant factors is strongly suggested to be important (Mise et al. 1993). During this study, systemic infection of the virus was found in approximately 10% of cowpea individuals inoculated with chimeric CCMV, in which the MP gene of CCMV was replaced by the MP gene of BMV. Progeny viruses obtained from these infected individuals systemically infected all newly inoculated cowpea individuals, strongly suggesting the emergence of adaptive mutants (Mise et al. 1993). Sequencing of these chimeric CCMV progeny viruses revealed that site-specific nonsynonymous lysine or arginine substitution mutations in the central region of the BMV-MP gene were required for the acquisition of systemic infectivity in chimeric CCMV (Fujita et al. 1996; Sasaki et al. 2001). Because it was difficult to incorporate the green fluorescent protein (GFP) gene into the genome of BMV while maintaining infectivity, monitoring of the virus in infected leaves required in situ hybridization of viral RNA in detached cowpea epidermis (Mise and Ahlquist 1995) and histochemical detection by immunostaining of tissue-fixed vertical sections of inoculated leaves with anti-BMV antibodies (Fujita et al. 2000). These techniques revealed that chimeric CCMV spread cell-to-cell in inoculated leaves to some extent, but that infection is restricted at the stage of invasion into vascular tissues. In other words, it was shown that the incompatibility of BMV-MP to cowpea factor in these tissues can be overcome through by mutation of amino acid residues at specific sites in BMV-MP (Sasaki et al. 2001).

Plant viruses can be classified into two groups based on whether they require CP or not, although MP function is essential for cell-to-cell movement. We found that the MP gene of each virus determines the requirement for CP in the cell-to-cell movement of BMV, which requires CP, and CCMV, which does not (Sasaki et al. 2003), that the requirement for CP varies depending on various mutations in the MP gene (Takeda et al. 2004, 2005), and a new strain of BMV that does not require CP for cell-to-cell movement was discovered (Takeda et al. 2005). These findings suggest that plant viruses can select their mode of movement to infect specific plants and can change their host range. In parallel with the above studies, we investigated various functions of BMV MPs, and found that BMV MPs bind to single-stranded nucleic acids without sequence specificity (Fujita et al. 1998), localize in plasmodesmata that connect cells (Fujita et al. 1998), and co-localize with replication enzyme component proteins 1a and 2a (Dohi et al. 2001) and phosphorylated and interacted with CP in infected cells (Akamatsu et al. 2007). Furthermore, the C-terminus of BMV CP was shown to play a role in virus movement as a viral factor other than MP (Okinaka et al. 2001).

Analysis of host plant factors involved in bromovirus infection in plants

Thus, much has been learned about the viral factors involved at the level of intercellular movement, particularly MP of BMV, with some progress in the study of plant factors. In transgenic *N. benthamiana* plants in which the expression of *NbNACa1* was down-regulated by silencing, the efficiency of cell-to-cell movement of BMV was reduced and the efficient localization of MPs in the cell wall was inhibited (Kaido et al. 2007). In addition, HCP1, which was identified in barley as a host factor that interacts with BMV CP, showed a positive correlation between the binding ability of various mutants of CP and HCP1 and the ability of BMV with these CP mutations to infect plants (Okinaka et al. 2003). Although the involvement of the identified host factors in viral infection was suggested, it was difficult to comprehensively analyze the host factors involved in bromovirus infection in detail because genomic information on BMV host plants had not been reported at that time, and genetic analysis using mutant plants was not easy.

Therefore, we sought to use BMV and other bromoviruses as direct research targets or models to analyze the infection mechanisms of BMV and other bromoviruses in their original hosts, plants, using BMV, which has led research on viral RNA replication mechanisms using in vitro RNA synthesis systems derived from infected plants, protoplast inoculation systems, and budding yeasts. To analyze the infection mechanisms of BMV and other bromoviruses, plants other than cowpea and barley were needed to be used. We found that a bromovirus SBLV efficiently and systemically infects the model plant *Arabidopsis thaliana* (Fujisaki et al. 2003). We also found that SBLV induces severe systemic necrosis when infects certain ecotypes, and that the development of necrosis is regulated by the semidominant locus *SSBI* (Fujisaki et al. 2004). On the other hand, BMV cannot infect *Arabidopsis*. We inoculated various existing mutant *Arabidopsis* plants with BMV and investigated its effect on infection, and identified a plant gene, *CPR5*, that negatively regulates BMV infection (Fujisaki et al. 2009). The mutant *cpr5* showed increased resistance to filamentous fungi and bacteria, but decreased resistance and showed susceptibility to BMV. One of our recent studies has also

provided interesting results suggesting that BMV utilizes two of the reactive oxygen species (ROS) that positively regulate plant immunity for its replication (Hyodo et al. 2017).

Conclusion

Recently, it was reported that a specific strain of BMV infects rice (Ding et al. 2006). Rice is an important crop, and at the same time, it is a model plant that is relatively easy to create recombinants due to the availability of genome information, various varieties, mutants, and recombinant lines. We inoculated various rice varieties with several BMV strains and tested their infectivity. As a result, we have successfully isolated two genes classified into *NLRs*, which are known as major resistance genes in many plants (Mise et al. 2023). We are now in the process of analyzing in detail how the NLR receptor protein, the product of the genes, recognizes BMV factors and triggers resistance. We have also determined the draft genome sequence of quinoa, a test plant for plant viruses and a functional crop (Yasui et al. 2016). We will continue to promote basic research for the establishment of new disease control technologies by adding studies on BMV-rice and BMV-quinoa interactions to our existing research.

Acknowledgements I would like to express my sincere gratitude to the late Prof. Jiko Shishiyama for giving me the opportunity to start my career in plant pathology and my deepest gratitude to the Emeritus Prof. Iwao Furusawa for guiding me in my research on bromoviruses and for his enthusiastic encouragement for many years. I would like to express my deep gratitude to Prof. Paul Ahlquist at the University of Wisconsin, USA, who gave me the opportunity to start host specificity research of bromoviruses while I was studying in the USA and has continued to inspire me with his pioneering and leading research on bromovirus replication after my return to Japan, and the late Prof. Yoshimi Okada for his support during my study in Prof. Ahlquist's lab. I also express my gratitude to Dr. Tetsuro Okuno, Dr. Naoto Yamaoka, Dr. Yasuyuki Kubo, Dr. Yukio Tosa, Dr. Mamoru Horikoshi, Dr. Seiji Tsuge, Dr. Hitoshi Nakayashiki, Dr. Yoshitaka Takano, Dr. Masanori Kaido, and Dr. Akira Mine for their various advice and support in advancing this research. I would also like to express my sincere gratitude to Dr. Kappei Kobayashi, Dr. Yasufumi Hikichi, and Dr. Masashi Mori, with whom I shared many hardships from the start of the experimental system in the Laboratory of Plant Pathology, Kyoto University (KUPP), when I started molecular biological studies of plant viruses. I am grateful to Drs. Yasunari Fujita, Miki Fujita, Yasushi Okinaka, Koji Dohi, Nobumitsu Sasaki, Koki Fujisaki, Atsushi Takeda, Naoko Akamatsu-Kobayashi, Fukumatsu Iwahashi, Taiki Narabayashi, Hiro-oki Iwakawa, Kiwamu Hyodo, and all the students, researchers and staff in KUPP, for their support and cooperation in this research. Most of this research was supported by Grant-in-Aid for Scientific Research on Priority Areas (Dr. Ichiro Uyeda) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), and several competitive research funds from Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS). I would like to express my gratitude to them. Finally, I would like to express my deepest gratitude to my family for supporting my research life for many years.

Declarations

Conflict of interest The author declares no conflict of interest.

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