Begomovirus DNA replication and pathogenicity

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Begomoviridae is the largest genus of the family of single stranded DNA plant viruses, Geminiviridae and is responsible for significant agro-economic losses worldwide. Its small single-stranded DNA genome primarily replicates by rolling circle replication (RCR) mode with the help of certain viral and host factors. The role of virus encoded Rep protein in initiation and immediate post-initiation phases of RCR has been the subject of various studies. We have identified many host proteins which interact with Rep protein of a member of Begomovirus, namely, Mungbean yellow mosaic India virus, thereby playing a role in viral DNA replication. Of these, the role of host RAD54 protein is significant as the rad54 mutant of Arabidopsis does not permit mini viral DNA replication. The plant hosts protect themselves from begomoviruses by activating RNA interference (RNAi) pathways targeted against the viruses. However, the virus can also sometime overcome this form of host defence by encoding RNA silencing suppressors, which attenuate host RNAi and are regarded as major pathogenicity determinants. The viral suppressors do not share any signature sequences and are structurally and mechanistically dissimilar. These can be detected effectively, only through specialized functional assays. In this review, we also point out the potential biotechnological applications of the suppressors and discuss about various possible containment strategies for begomoviruses, including an exciting new approach involving artificial micro-RNAs.

Keywords: Artificial microRNA, Begomovirus, DNA replication, RNAi-suppressors.

Introduction

VIRUSES have emerged as major plant pathogens as 47% of all emerging infectious diseases of plants have been reported to be caused by viruses[1]. Plant viruses are divided into more than 15 families, of which *Geminiviridae* constitutes the second largest family. Geminiviruses are characterized by their twin geminate particles, ssDNA circular genomes and whitefly (*Bemisia tabaci*) mediated transmissibility (Figure 1). Around 25 years ago, when genomic properties of geminiviruses were studied, many scientists regarded them as 'friends of humans', because of their potential as gene transfer vectors in plant genetic engi-

neering and non-harmful effects on host plants. But far from being friends, these viruses have now emerged as foes and are a serious threat to world agriculture now. Increase in international commodity trade, intercontinental transportation networks and a changing global climate have contributed to the spread of this virus and its whitefly vector[2]. *Geminiviridae* is represented by four genera namely *Mastrevirus*, *Curtovirus*, *Topocuvirus* and *Begomovirus*. The genus *Begomovirus* is the largest and consists of more than 180 species and several unassigned isolates[3]. They are causal agents of some of the most devastating plant diseases like leaf curls in cotton, pepper and tomatoes, mosaic and yellow mosaic of cassava, pulses and beans. During severe infection, *Mungbean yellow mosaic India virus* (MYMIV) and *Tomato leaf curl virus* (ToLCV) can damage up to 70% and 100% of yield respectively[4]. A 1992 estimate puts annual loss in India due to MYMIV alone at US$ 300 million. Both MYMIV and ToLCV are being used as models in our laboratory to unravel several key biological aspects of begomoviruses.

Detection of begomoviruses

The first and foremost task in begomovirus research is the detection of virus or viral DNA. The prevailing techniques, based on PCR and viral serology, are constrained by availability of prior sequence information and virus-specific antibodies. Moreover, these techniques are getting handicapped in the wake of fast evolving characteristics of the single-stranded viral genomes. To overcome these problems, many laboratories including our own routinely employ a rolling circle amplification (RCA) technique, which uses a high fidelity φ 29 DNA polymerase along with random hexamers to detect the genomes of various begomoviruses. Using this method, our laboratory has identified genomes of begomoviruses from more than 20 different sources – ranging from Sponge gourd to *Jatropha*. This method can be adopted to develop high throughput diagnostic kits for quarantine purpose.

Aspects of begomovirus DNA replication

Since the time begomoviruses were discovered, attempts have been made to unravel viral DNA replication mechanism. Dissecting the viral genome and its biochemistry of replication gives us a great insight into the organization and functional aspects of this virus and thus generate the hope to interfere with the viral multiplication processes.
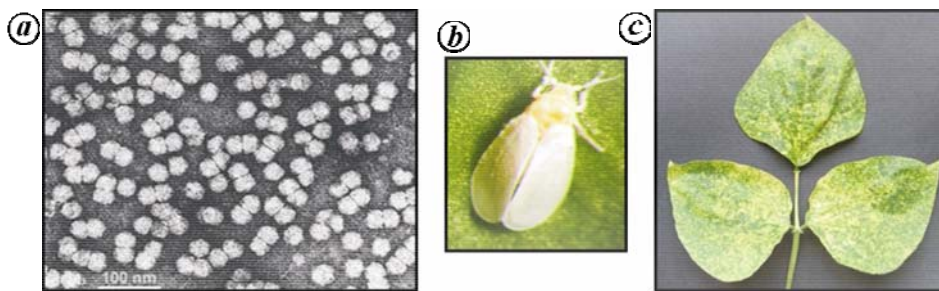
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Figure 1. Structure, vector and symptom of begomovirus infection. ***a***, Electron micrograph showing the twinned structure of begomovirus virions (adapted from http://www.ncbi.nlm.nih.gov/Images/em_gemin.jpg). ***b***, White fly (*Bemisia tabaci*) transmits begomovirus causing mosaic and curling symptoms in infected plants (adapted from http://www.ag.arizona.edu/horticultura0304fig1.jpg). ***c***, Mungbean plant leaves showing typical yellow mosaic symptom caused by MYMIV[37].
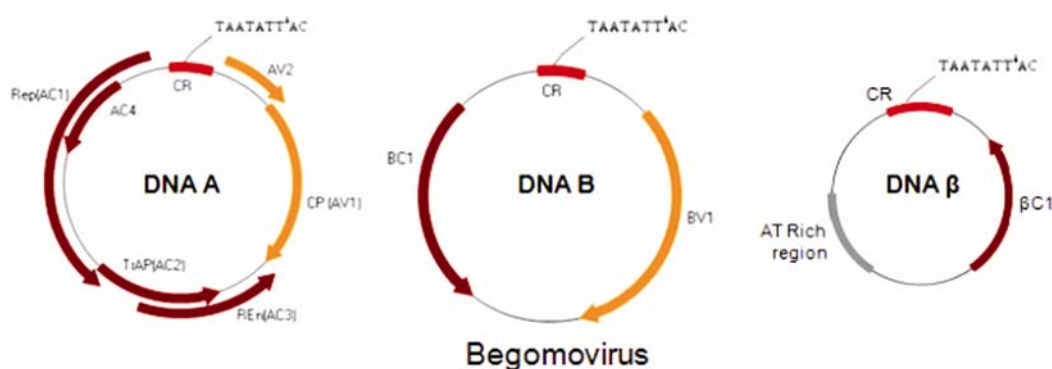

Figure 2. Genome organization of begomovirus. Genome maps of type species of begomovirus – DNA-A and DNA-B components represents bipartite genome of MYMIV. Satellite DNA-β is often associated with DNA-A comoponent of monopartite genome of say, ToLCV. DNA-A component encodes for: Rep/AC1, replication initiator protein; TrAP/AC2, transcription activator and/or called silencing suppressor; REn/AC3, replication enhancer; CP/AV1, coat protein. DNA-B component encodes for: MP/BC1, movement protein; NSP/BV1, nuclear shuttle protein. βC1 protein of DNA β performs the role of RNAi suppression in monopartite begomoviruses. (Photo courtesy: Thesis, Kalyan Kumar Pasumarthy.) The location of the conserved region of all geminiviruses, namely, TAATATTAC has been indicated.

Genome organization

Begomoviruses have the most complex genome organization among all sub-groups of geminiviruses. In a majority of the cases, the viral genome is bipartite, and comprises two separately encapsidated genome components, DNA-A and DNA-B (e.g. in MYMIV). However, in a few cases, the genome is monopartite (e.g. ToLCV). A satellite DNA, known as DNA β, has sometimes been observed to be associated with some of these monopartite species (Figure 2), and is considered essential for induction of disease symptom[5]. The DNA-A and DNA-B are similar in size (2.7–3.0 kb), but differ in sequence, except for a 200–250 bp region of high sequence homology known as the common region (CR). The CR region is a part of large intergenic region (IR) – that contains origin of replication and divergent promoters for transcription.

DNA-A component of begomovirus contains five to six ORFs which can encode for proteins of more than 10 kDa size. These proteins can be classified into three groups:

DNA replication initiation proteins – AC1/Rep (replication initiator) and AC3/REn (replication enhancer); host gene regulation and silencing suppressor proteins – AC2/TrAP (transcription activator), AC4 and the pre-coat protein AV2; viral assembly protein – AV1/CP (coat protein)[5]. Similarly, the DNA-B component contains two ORFs which encode for proteins that assist in intra-cellular and inter-cellular viral movement – BC1/NSP (nuclear shuttle protein) and BV1/MP (movement protein).

The predominant mode of begomovirus DNA replication is rolling circle replication (RCR). Though both the DNA components of begomoviruses replicate by RCR, only DNA-A can replicate independently, i.e. in absence of DNA-B, but the same independence does not hold true for DNA-B. To enhance our understanding of begomovirus DNA replication mechanism, we selected MYMIV DNA 'A' to serve as a model system, mainly because of the economic impact of the virus. Subsequent to whitefly-mediated transmission, the viral genome is introduced into the nucleus and it initiates a three-stage DNA replication process (Figure 3). In the first stage, in conjunc-
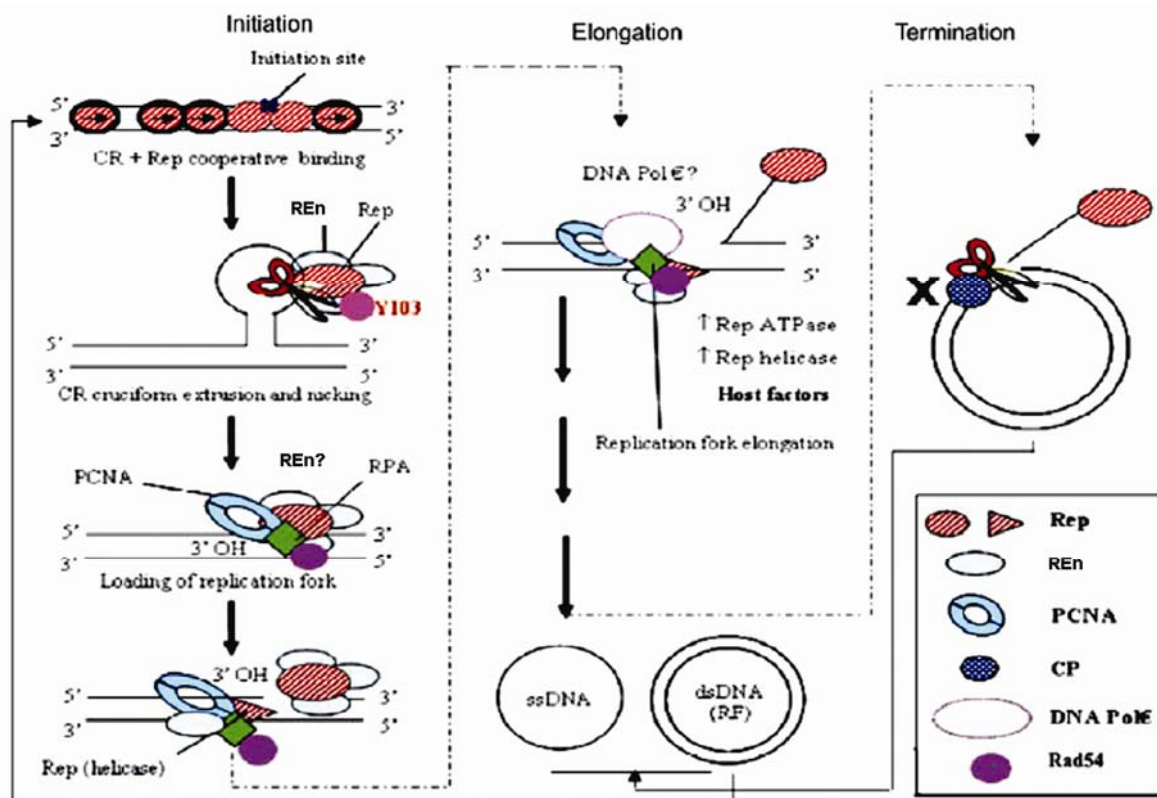
Figure 3. Model for begomovirus rolling circle DNA replication. *Initiation* – During replication initiation, the Rep protein binds CR region of DNA 'A' in a cooperative manner, and eventually nicks the DNA at the specified sequence to initiate RCR. The Y103 amino acid of Rep is essential for nicking. The helicase activity of Rep protein then might help in melting the origin further, allowing other host factors such as RF-C, PCNA, RPA, RAD54 and DNA polymerases, etc. to associate at the 3′ OH end of the nick to form a progressive replication fork. *Elongation* – During progression, many other host factors might participate to regulate the replication rate. *Termination* – At the termination step of RCR, the Rep protein again cuts and religates the newly synthesized ssDNA to eventually generate many copies of viral ssDNAs[38].

tion with only the host cellular factors, viral circular ssDNA is converted into supercoiled dsDNA intermediates or replicative forms (RF). The resultant dsDNA is transcriptionally active and leads to the transcription of various viral factors. The second phase begins with the dsDNA acting as a template to synthesize ssDNA using the viral as well as host cellular factors via the RCR mode. The newly synthesized ssDNA can also re-enter the DNA replication pool. This ssDNA provides foundation for viral assembly and/or viral spread in the next stage. Besides RCR, recombination-driven replication (RDR) mechanism also contributes to the viral DNA synthesis to some extent[6].

Characterization of MYMIV Rep and viral DNA replication

As discussed earlier, the key to MYMIV DNA replication is conversion of circular ssDNA to transcriptionally active dsDNA intermediate. The dsDNA transcribes replication-related proteins such as Rep which is a critical viral pro-

tein to initiate and extend RCR. Rep is a multifunctional, oligomeric protein which possesses site-specific DNA-binding, nicking and ligation properties, and executes ATP-dependent topoisomerase I, ATPase and helicase activities[7,8]. In order to initiate RCR, Rep recognizes the CR and this recognition is facilitated by the presence of four highly sequence-specific Rep binding sites (iterons). The CR shows the highly conserved stem loop structure keeping the geminivirus-signature nonamer sequence (TAATATT ↓AC) in the loop. In order to initiate rolling circle replication, Rep nicks the DNA between 7th and 8th nucleotide (arrow) in the nonamer sequence[7].

In our laboratory, the sequence-specific binding characteristics of MYMIV-Rep to its cognate CR DNA are confirmed by the filter binding, EMSA and DNase I foot-printing assays[9]. Another unique feature of MYMIV origin of replication is the presence of bipolar iterons (CGGTGT), i.e. on both sides of the stem loop structure. As revealed from DNase I foot-printing assay, Rep binding to the four iteron sites takes place in a cooperative manner[9]. The specific DNA binding activity of Rep is located at the region spanning the N-terminal 133 amino

acids of MYMIV-Rep. As the Rep binds to the cognate CR DNA, it causes certain structural distortions in the loop-region around MYMIV origin of replication. DNase I footprinting experiments and $KMnO_4$ oxidation-based probing suggest formation of cruciform structure at the replication origin as a result of MYMIV-Rep binding. It is also likely that some host factors participate in this initiation process to control the rate characteristic of the *in vivo* RCR process of viral DNA.

Subsequent to the Rep-mediated initiation, the elongation phase of DNA replication takes place from the free 3′-OH end of the nick created by Rep. During the elongation phase, various host fork proteins assemble at the free 3′-OH end to extend the primer in the 5′ → 3′ direction. At this stage, the role of a replicative helicase to dislodge the existing viral strand by DNA unwinding is most important for the synthesis of new viral strand. Our laboratory showed for the first time that MYMIV-Rep possesses characteristic motifs of a helicase enzyme and it also functions like a helicase with limited processivity[8]. The Rep helicase translocates in 3′ → 5′ direction and for its proper activity, it requires the ssDNA of more than six nucleotides. The MYMIV-Rep protein with a mutation in the oligomerization domain fails to show the helicase activity.

The anatomy of the replication fork has not been well-characterized so far. As MYMIV DNA replication elongation is completed, the nascent concatenated DNA undergoes Rep-mediated nicking and ligation for the formation of single-stranded circular viral DNA. These ssDNA genomes re-enter the replication process and eventually produce a large number of ssDNA copies in the host cell.

Role of host factors in begomovirus DNA replication

The role of viral Rep and REn in initiation, elongation and termination processes of MYMIV genome replication has been studied to some extent. However, the presence of host replication factors is essential for execution of all of the steps mentioned above. A vast majority of begomoviruses infect terminally differentiated cells which have exited cell cycle and have a low concentration of DNA replicative enzymes. To counter this adversity, the Rep protein of the virus interacts with host factors to create a favourable S-phase like cellular environment. Various host factors and their roles in viral DNA replication and maintenance are being explored gradually.

As the virus encoded Rep protein is the key player for viral DNA replication, Rep interacting host factors contribute significantly to the replication process. Using a variety of interaction approaches, the Rep protein has been found to interact with a large repertoire of host factors such as pRBR to alter cell cycle[10,11], RF-C for loading of PCNA[12], histone H3 for putative removal of nucleosomal block and efficient transcription and replication[13], GRIMP (kinesin) and GRIK (a Ser/Thr kinase) for mitotic activity, etc.

The interaction studies of MYMIV-Rep with the host peptide library in phage display and host cDNA library in yeast, carried out in our laboratory, have demonstrated that Rep might interact with a wide range (about 150) of host factors. We found that MYMIV-Rep – PCNA (proliferative cell nuclear antigen) interaction down regulates nicking/closing and ATPase activities of Rep[14]. MYMIV-Rep is shown to interact with the middle subunit of the host replication protein-A (RPA) which is a hetero-trimeric, single strand DNA binding protein[15]. This interaction leads to down regulation of nicking/closing activity of Rep, but enhancement of the ATPase and helicase activities. Recently, we have characterized an important factor, namely the conserved Rad54 protein, which has been found to support MYMIV-DNA replication in yeast-based model system[16]. The biochemical analyses have shown that RAD 54 enhances the intrinsic ATPase and the helicase activities of Rep, which might be important in the post-initiation steps of the viral replication. The role of RAD 54 in MYMIV-DNA replication has also been demonstrated by studying the *in vitro* replication of plasmid bearing MYMIV genome using the *Saccharomyces cerevisiae* wild type and *rad54* deficient nuclear extract. The *in vivo* studies with *Arabidopsis* wild type and mutants also show the important role of Rad54 protein in viral DNA accumulation (Figure 4). It is noteworthy that we have validated the requirement of two dozens of host factors for MYMIV-DNA replication using the above mentioned yeast model system.

A strong interaction between MYMIV-Rep and MYMIV-CP has also been demonstrated by *in vivo* and *in vitro* studies[17]. This interaction causes downregulation of Rep activity, specifically nicking. Thus, by blocking initiation of RCR and thereby the production of ssDNA, MYMIV-CP might play a role in limiting the viral DNA copy number.

The begomoviral DNA replicates rapidly in the nucleus leading to the generation of thousands of viral ssDNA circles. These ssDNA associate with coat protein to form virions or form complexes with virus encoded nuclear shuttle protein (NSP). The ssDNAs are exported out of the nucleus into the cytoplasm with the help of NSPs from where they are destined for cell-to-cell systemic spread through plasmodesmata with the help of viral movement proteins. The end result of mobilization and amplification of the viral genomic DNA is the production of begomovirus infection symptoms and disease.

Pathogenicity aspects

Plants respond to viral attacks in a variety of ways. However, unlike mammals, they lack protein-based adaptive
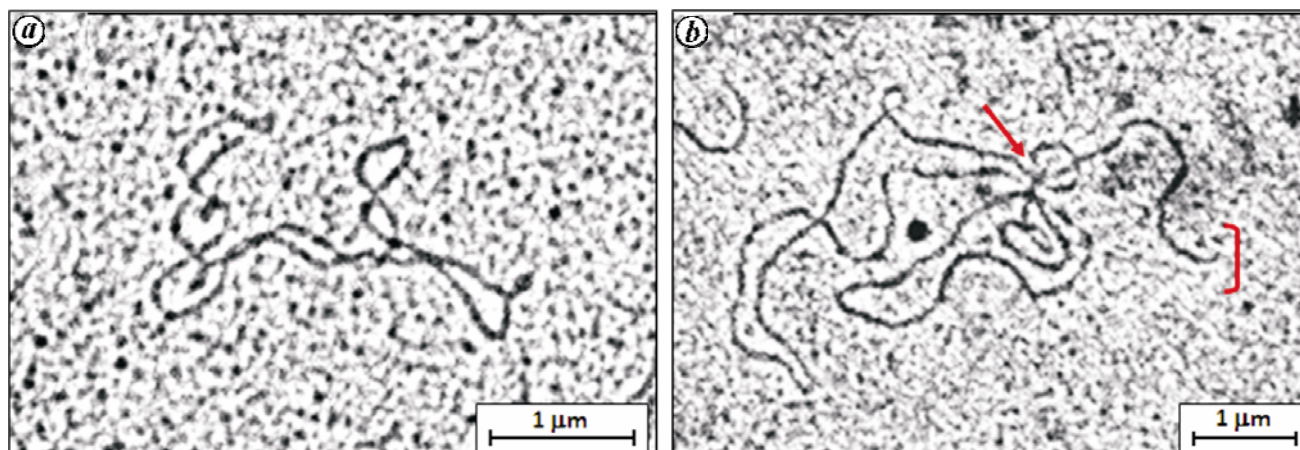
Figure 4. EM analysis of the products of the *in vitro* replication assay. Electron micrographs showing (*a*) the unreplicated supercoiled YcpO⁻-2A DNA (derived from ARS removed Yeast shuttle vector YCp50 which contains two tandem copies of MYMIV DNA-A component) and (*b*) *in vitro* replicated rolling circle intermediate of YcpO⁻-2A DNA. In panel (*b*), the terminal and initial points of the 'tail' (representing the replicated zone) are marked by the red symbols → and] respectively. Contour length measurement reveals the extent of replication of the molecule to be ~15.6%. Out of 200 molecules of the *in vitro* reaction products, seen in a typical electron microscopic field, only 30% of the molecules (~60 molecules) show the lariat structure with varied extension of the tail. (Courtesy: Nirupam Roy Choudhury, International Centre for Genetic Engineering and Biotechnology, New Delhi, India.)

immune system and instead, heavily rely on the ancient nucleic acid based RNAi pathway. As a counter strategy, viruses encode factors to suppress RNAi. These factors often control pathogenicity as these convert weak pathogens to the virulent ones, and absence of these debilitate the viruses for growth, and thus they are also known as pathogenicity determinants.

RNAi as antiviral mechanism

Mechanistically, RNAi is triggered by various forms of double-stranded RNA (dsRNA), which may be endogenous in the cell or encountered during various replicative phases of the invading virus. These dsRNAs are substrates for a class of RNase III type endoribonucleases, called Dicer/Dicer-like (DCL) enzymes, which cleave substrates into characteristic 20–25 nucleotide small RNAs, often called small interfering RNAs (siRNAs). The siRNA is recruited into a multi-protein RNA induced silencing complex (RISC), which cleaves target RNA, in a sequence-specific manner. Plants encode several forms of RNA dependent RNA polymerase (RdRP) and a subset of these are involved in siRNA amplification process which ensures further persistence and systemic spread of RNA silencing, even in the absence of the immediate dsRNA trigger[18]. The siRNA programmed RISC of plants has been figuratively compared with the scheme of mammalian T-cells harbouring a surface-bound immunoglobulin-like protein called T-cell receptor[19]. RISC finds the viral transcripts using a specificity adaptor – a siRNA, rather than a T-cell receptor – and then destroys it by cleaving, rather than eliminating the infected cell. Thus, the specificity determinant is derived directly from viral RNA, meaning

that the viral genome is turned against itself. Furthermore, the systemic relay of virus specific siRNAs to distant cells through plant vasculature spreads the news of infection and prepares the new cells to be ready with their RNA silencing defences in advance, thereby providing a mammalian style systemic immune surveillance mechanism.

Begomoviruses are also, both inducers and targets of RNAi. The begomovirus siRNAs are of 21, 22 and 24 nucleotide in length implying that all the four DCLs are involved in their generation[20]. Moreover, many segments of the viral DNAs also are methylated in a siRNA dependent manner in response to infection[21]. However, unlike the case in mammalian systems, the host microRNAs that interfere with replication and spread of plant viruses are not known yet.

Viral suppressors of RNAi as the major pathogenicity determinants

In response to plant antiviral RNA silencing, viruses are not behind in waging an arms race to neutralize host defences. They have evolved several RNAi evading mechanisms like evolution of siRNA resistant satellite genomes, defective interfering RNAs, loss of target sequences by high mutation rate, formation of RISC-inaccessible secondary structures, associating with protein complexes posing sterric hindrance, encapsidation and partitioning their replicative cycles in vesicles, chloroplasts and nucleus[22]. However, the most potent weapon to counteract silencing is evolution of specialized proteins, coded from the viral genomes, called RNA Silencing Suppressors. It is believed that these proteins have evolved inde-

pendently in various viruses as they do not share any common signature sequences and are structurally as well as functionally dissimilar from each other. The first clue about suppressors came from observations on 'synergism' where disease severity from one virus was found to exacerbate in presence of co-infection by a second unrelated virus. This led to the discovery of potyviral Helper component proteinase (HcPro) as the synergism determinant in potex–potyvirus interaction, which was subsequently proved to be a silencing suppressor[23,24] along with *Cucumber mosaic virus* (CMV) 2b protein[25]. Since, HcPro and 2b were previously characterized as pathogenicity determinants, a re-investigation of pathogenicity factors from diverse viruses was carried out which revealed that many were indeed silencing suppressors[26]. Ours and other laboratories have confirmed that begomovirus AC2, AC4, AV2 (pre-coat protein) and NSP are also such silencing suppressors and pathogenicity determinants[27]. The AC2, AC4 mutants of MYMIV can hardly replicate both in the yeast as well as *in-planta* model as these suppressors protect viral transcripts from host-mediated degradation.

Detection of suppressors

Screening of viral ORFs for suppressor activity is of utmost significance and it demands development of assay systems which are quick to perform, easy to reproduce and work well across all viral families including plant and animal viruses. Our laboratory has developed six such detection techniques. The first technique is based on the widely used reversal of silencing in GFP-silent transgenic tobacco lines[27] (Figure 5). Another assay utilizes the principle of virus induced gene silencing (VIGS)
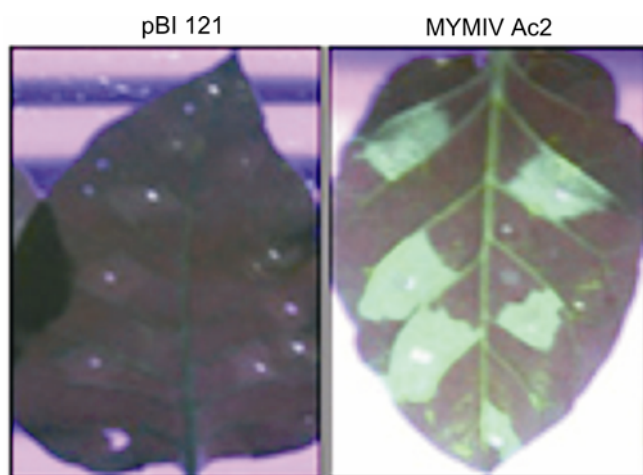

Figure 5. Reversal of silencing assay showing strong suppression activity of MYMIV-AC2. The ectopic expression of RNAi suppressors turns on the green colour of GFP in the tobacco leaves which show the red auto-fluorescence otherwise. pBI121 is the empty vector and was used to clone AC2 ORF of MYMIV. The UV lamps (pink appearance) beneath the leaves are also shown.

and tracks the enhancement of an amplicon (episome) based on RCR of a begomoviral replicon in planta[27]. The third assay is based on effect of suppressor on *de novo* RNA silencing introduced through a hairpin GFP construct, unlike pre-established silencing of the first technique. One more assay is carried out using insect Sf21 RNAi sensor lines[28]. Other assays utilize the *in vitro* dicing and slicing reaction competence of wheat germ extract. These assays are not only helpful in identification of various suppressors but also provide hints towards probable mechanism of suppression. Using these assays the AC2 ORF of a dozen different begomoviruses from different parts of India were characterized for their suppressor activity and strength of suppression using time-kinetics. These assays have also been used to identify the suppression activity of 7a ORF of SARS virus, B2 ORF of *Flock house virus* (FHV) and other animal viruses, indicating versatility of these assays.

Mechanism of suppression

Suppressors are known to target different stages of the RNAi pathway. CMV-2b specifically blocks Ago1 cleavage activity to inhibit RNAi and is also involved in interfering with the microRNA pathway[29]. In contrast, Hc-Pro and tombusvirus P19 hijack and stabilize miRNA/miRNA* duplexes, preventing their loading into the RISC complex[30,31]. FHV-B2 protein acts like an inhibitor of siRNA biogenesis and interacts with the 'PAZ' domain of the Dicer[28]. The real mechanism of begomoviral AC2 suppressor has remained elusive so far. Recent findings in our laboratory have unravelled this mystery. The MYMIV-AC2 is found to be a transactivator but the domains of suppression and transactivation are separable. The full length AC2 protein is biochemically a multimer, inhibit siRNA generation in plants and interacts with many *Arabidopsis* proteins including those involved in RNAi pathway namely, AGO1, RDR6, DCL1 and ARF6 as evidenced in the phage display as well as yeast two-hybrid assays. Both, MYMIV and ToLCV AC2 inhibit the enzyme activities of host RDR-6 and AGO1 proteins, which are important in initiation and effector activities of RNAi.

Biotechnological application of suppressors

Suppressors can reverse gene silencing effects and allow high transgene expression – a desired goal of molecular farming. Genetic crossing of a TEV Hc-Pro suppressor line with a PVX-GUS amplicon line resulted in a dramatic increase in GUS expression to the tune of 3% of total soluble protein[32]. In agro-infiltration based transient expression systems, expression of a range of proteins was enhanced 50-folds or more in the presence of p19 so that protein purification could be achieved from as little as
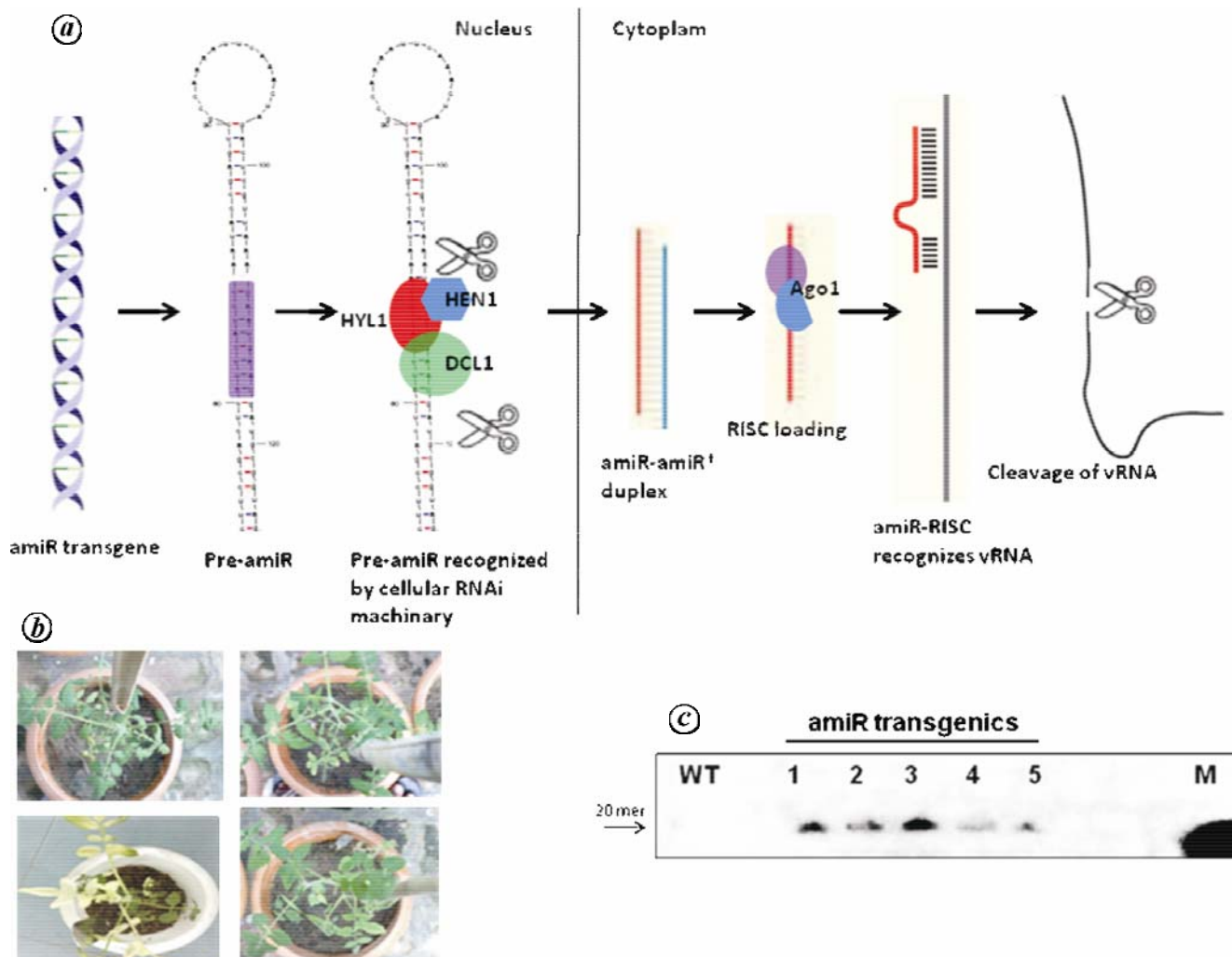
Figure 6. AmiR engineering: *a*, A model depicting processing of pre-amiR and consequent silencing in plants. The synthetic amiR gene is stably incorporated into the plant genome, which transcribes to generate amiR precursor. Because of identical secondary structure of pre-amiR with endogenous pre-miRs, the former is easily recognized by cellular RNAi machinery and is sequentially processed into mature amiR. AmiR is loaded to RISC complex and amiR–RISC recognizes and makes viral RNAs (vRNAs) dysfunctional. *b*, AmiR overexpressing tomato transgenics and (*c*) production of mature amiRs in transgenic plants as detected by small RNA Northern hybridization. M: Marker; 1–5: tomato transgenic expressing amiR; WT: wild type tomato shows no amiR expression.

100 mg of infiltrated leaf material[33] (US Patent 7217854). Our laboratory has demonstrated the strength of MYMIV-AC2 in molecular farming by showing that the introduction of AC2 in a GFP silent tobacco transgenic line by means of genetic crossing fully reactivates the silent model reporter gene GFP. The potential of AC2 in recovery of the silencing induced plant diseases, like bleaching of chlorophyll, stunting in growth, etc. is being currently tested.

Containment of begomoviruses

In the arms race between the begomoviruses equipped with RNAi suppressors and their hosts with antiviral RNAi, the former seems to be having an edge, at least as seen from the human angle. The enormous loss of our crops to

begomoviral diseases necessitates development of intervention strategies to efficiently contain the virus. Spray of insecticides to get rid of the virus transmitting whitefly vector, is neither an effective nor an eco-friendly approach. Unfortunately, stable natural resistance sources for begomoviruses like MYMIV and ToLCV are few and plant breeders have not been successful in introgressing these largely multi-genic traits into elite cultivars. Hence, modern biotechnology needs to offer an attractive alternative of engineering begomovirus resistance through transgenic route[4]. Pathogen-derived resistance (PDR) through the expression of various full length/truncated or defective viral proteins like Rep mutants of maize steak virus has been achieved. Antisense and RNAi technology has also been used but without consistent success. An exciting new approach using another set of small RNAs called

artificial microRNAs (amiRs) to achieve virus resistance has been proved successful for few viruses[34]. This approach has not been used on geminiviruses so far. The Pre-amiRs, designed and synthesized in our laboratory are not only consistently processed into small RNA (microRNA) species, in *in vitro* assays that use the *Arabidopsis* inflorescence and wheat germ extracts, but also in stably transformed tomato plants (Figure 6). These small RNAs are designed to target the viral Rep and suppressor transcripts. The efficacy of these transgenics in resisting the ToLCV is being tested currently.

Apart from these strategies many other approaches like use of ribozymes, DNA binding proteins, peptide aptamers, GroEL, etc. have been attempted. A hammerhead ribozyme directed against Rep showed ~33% cleavage activity on synthetic *rep* transcript[35], while the *Bemisia tabaci* GroEL gene, expressed in transgenic tomatoes under the control of a phloem-specific promoter, protected the plants from yellow leaf curl infection[36]. All these strategies have their share of advantages as well as disadvantages and it seems that stacking together of multiple approaches would only provide a durable resistance against begomoviruses, given their extremely high penchant for rapid mutation and recombination.

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