boson which is meant to transmit a short-ranged force can be made to acquire mass. Thus, the unwanted Goldstone boson is 'fed' to the gauge boson, which becomes massive and is capable of mediating a short-range force.

In the electroweak gauge theory there are four gauge bosons. Assuming the gauge symmetry to be spontaneously broken and appealing to the Higgs mechanism, it is possible to keep the electromagnetic field quantum γ massless and the three weak bosons $W^{\pm}$ and Z massive. It is the massive nature of the W and Z which makes the 'weak' force weak. To put it differently, the Higgs mechanism gives us a way to create the asymmetry between the left-handed and the right-handed particles – an asymmetry which is manifest as the well-known parity violation in weak interactions.

But what is the price for invoking the Higgs mechanism in the SM? After making the three weak bosons massive, one component of the original neutral ϕ field is left over. This relic should be existing as a neutral scalar particle with unknown mass, and this is the Higgs boson of the SM. Its characteristic feature is that its coupling to a fermion pair is proportional to the fermion mass. Present data rule out the Higgs boson lighter than about 60 GeV.

Supersymmetry. It proposes a spin-doubling of the elementary fields: a fermion is associated with a boson of equal mass, and vice versa. Thus, an electron is associated with 'selectron' having zero spin, a quark with a spinless 'squark', a photon with a 'photino' having spin 1/2, etc. So far there is no shred of evidence for such particles in the experiments. Signals for even broken versions of the supersymmetry are not visible in the available experimental data.

String theory. In quantum field theory we deal with point particles. In the string theory the fundamental objects are one-dimensional entities called strings. In the laboratory the stringiness of the particles will not be evident because the string length is extremely small (of the order of Planck length ($\sim 10^{-33}$ cm); a string to an atomic nucleus is smaller than the nucleus to a mountain). As of now, string theory has not given any verifiable consequence nor explained any observed fact in particle physics.

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Monoclonal antibodies in the study of architecture of plant viruses

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Monoclonal antibodies have been used as probes to study the architecture of several plant viruses over the past decade. These studies complement the information obtained through X-ray crystallography and help in delineating epitopes on the surface of the virus. The monoclonal antibodies that recognize distinct epitopes also aid in unravelling the mechanisms of assembly/disassembly of virus particles. Group-specific and strain-specific monoclonal antibodies are widely used in the classification of viruses. The significant developments made in this emerging area are reviewed here with specific examples.

VIRUSES, a term coined by Beijerinck and Baur[1,2] to describe the causative agents of certain plant diseases, are obligate parasites that cause considerable damage to plants. Viruses are broadly classified on the basis of their particle morphology as helical, icosahedral or complex. There are about 33 groups of plant viruses, of which 22 are icosahedral, 9 are helical and 2 are complex[3].

Over the past decade the molecular architecture of these viruses has been studied using a variety of methods such as electron microscopy, X-ray low-angle scattering, single-crystal X-ray diffraction, sedimentation and other solution properties. An analysis of the three-dimensional X-ray structures of spherical plant, animal or insect viruses determined so far has shown that the viral coat protein most often has the same polypeptide fold irrespective of the origin of the virus. The coat

protein in these viruses is made up of a common 8-stranded β barrel motif. In spite of this common structural motif, these viruses are distinct with respect to their immunological cross-reactivity. Differences in immunological cross-reactivity are useful in the identification and classification of viruses.

Analysing the antigenic structure of a virus consists mainly in locating its antigenic determinants or epitopes, i.e. in ascertaining the accessible region on the surface of the virus recognized by the antibody molecules. There are various methods that can be used to map antigenic determinants[4,5]. These methods are particularly useful for understanding the architecture of viruses, for which a detailed three-dimensional structure has not been determined by X-ray crystallography. Immunological approach has been widely used in the case of plant viruses as a tool to investigate the structure and assembly. The results obtained for helical viruses such as tobacco mosaic virus (TMV) and potato virus Y (PVY) and icosahedral viruses like southern bean mosaic virus (SBMV), turnip yellow mosaic virus (TYMV) and physalis mottle virus (PhMV) are discussed in some detail in this review.

Antigenic structure of TMV

TMV is the type member of tobamoviruses and is found naturally throughout the world. In electron micrographs, it has the appearance of a rigid rod, 3000 Å in length and 180 Å in diameter, with a stain-penetrated central channel. A ssRNA molecule of 2×10^6 Da is encapsidated by 2130 identical coat protein subunits to M_r 17 K. Each helical turn of TMV particle consists of $16\frac{1}{3}$ protein subunits.

The primary structure of the TMV protein subunit was elucidated more than 30 years ago[6,7]. This was followed by an analysis of the antigen structure of the virus at molecular level[8,9]. The determination of the three-dimensional structure of TMV coat protein disc aggregate by X-ray crystallography[10] made it possible to interpret immunochemical data in terms of the known location of each residue. These pioneering studies led to the recognition of the relationship between antigenicity and amino acid properties of the residues constituting the determinant[11–15].

Seven continuous antigenic determinants were identified in TMV coat protein in the vicinity of the residues 1–10, 34–39, 55–61, 62–68, 80–89, 108–112 and 153–158 using polyclonal antibodies[14]. The length of these sequential epitopes (6–10 residues) corresponds to the size traditionally regarded as fitting that of the complementary antibody combining sites. Six out of seven of these antigenic regions were found to correspond to the major local maxima in a plot of temperature factor obtained by crystallographic refinement against residue number[16]. This correlation between high local mobility in the protein and the location of continuous epitopes was attributed to the fact that the mobility promotes complementary interactions between the epitope and the antibody site that is not optimally fashioned to fit the geometry of the epitope.

Altschuh *et al.*[15] identified a number of discontinuous epitopes on the surface of TMV particles by means of monoclonal antibodies (Mabs) raised against intact virions. About half of the eighteen antivirus Mabs they raised were so specific for the quaternary structure of the capsid that they were unable to bind to tryptic peptides of the coat protein.

Since immunization of mice with intact TMV particles precluded the formation of antibodies specific for the many hidden epitopes known to be present on adjoining subunit surfaces, it was necessary to study Mabs raised against monomeric subunits of the viral capsid. Such a study could establish whether Mabs raised against the subunits also preferentially recognized conformational features of the protein subunit that are absent in peptide fragments. Al Moudallal *et al.*[13] synthesized 18 peptides representing virtually the entire length of the polypeptide chain of TMV coat protein and analysed their ability to bind in an enzyme immunoassay to 30 Mabs raised against the dissociated viral subunits. Of these 30 Mabs, 23 Mabs could react with the whole virion as well as with the monomeric subunits. This suggested that majority of the Mabs to denatured proteins also recognized conformational features present on the intact virus. On the other hand, five Mabs could react only with the monomeric subunits, but not with the virion. These five Mabs could react with the peptides also, suggesting that they probably recognize regions that are buried in the intact virus. Two Mabs could react only with the virions but could not react either with the monomeric subunits or with the peptide fragments. These studies identified four new epitopes in the vicinity of residues 19–32, 90–95, 115–134 and 134–146. Thus, eleven continuous epitopes were identified in the TMV coat protein molecule, which showed that almost the entire surface of the molecule was antigenic.

Dore *et al.*[17] attempted to narrow down the location of the different epitopes by analysing the ability of the Mabs to cross-react with four tobamoviruses which had between 39% and 82% sequence similarity with type strain of TMV[18], and nine TMV mutant viruses or proteins which differed from type strain of TMV by one or two substitutions. By correlating the extent of cross-reactivity with the common accessible surface between the parent protein and each related protein, Dore *et al.*[17] could deduce that not every residue in the antigenically active peptide is a contact residue. It is interesting that the residues 72–77, 129–134 and 142–147, although highly accessible as visualized in X-ray studies, possessed no antigenic activity. This shows that the correlation between antigenicity and mobility is not solely based on high surface accessibility.

Monoclonal antibodies as probes for studies on assembly of TMV

Assembly of TMV is among the most well-studied self-assembly mechanisms[19]. TMV subunits aggregate to form highly polymorphic oligomeric structures depending on the ionic strength and the pH of the solvent[19,20]. One of the aggregates formed at neutral or higher pH is the two-layered protein disk. Each layer of the disk consists of 17 protein subunits organized with rotational 17-fold symmetry. This aggregate appears to be the most important intermediate in the assembly of the virus. A base paired loop structure in RNA approximately 1000 nucleotides downstream from the 3′ terminus inserts into the central cavity of the disk and initiates assembly[21] Both the 3′ and 5′ ends of RNA protrude from the same end of this initiation complex[22]. Binding of RNA brings about a change in the inter-protein subunit contacts from a stacked disk-like structure to a helix-like structure[23,24]. Further assembly takes place by addition of the disks to the free end, while the RNA is pulled through the central hole. As three nucleotides interact per subunit, approximately 100 nucleotides are protected from nuclease attack by the addition of each ring. Only the initiation step appears to have specificity, as the assembly can proceed with other RNAs to which the loop-like structure from TMV has been engineered[25].

Dore *et al.*[26] showed by electron microscopic studies that all Mabs which recognize intact virus as well as coat protein subunits bind to one end of TMV rods. The RNA molecule is oriented within TMV particles such that its 5′ and 3′ ends are at opposite ends of the helical particle. In order to distinguish the end to which the Mabs bind specifically, the virus particles were partially uncoated by the action of 6 M urea, a procedure which uncovers RNA tails at the 5′ terminus[27]. By using electron microscopy, which permits the visualization of both the RNA tails and the gold-labelled Mabs, it was shown that these antibodies specifically recognized the extremity of the virus particle containing the 5′ end of the RNA[28]. In view of the orientation of the RNA molecule inside the helical virus[29,30] rod, this observation suggested that the antibodies bind to the bottom surface of the subunit.

These antibodies were then used to determine if short rods of stacked disks of viral protein were polar, i.e. if all the successive rings were facing the same way. The polar orientation, which was presumed for the postulated transformation of two-layer disks into polar helical rods[31–33], would require that opposite subunit surfaces be exposed at the two ends of the stacked disks, in which case, Mabs should bind to only one end of the stacked disks. Contrary to this expectation, Dore *et al.*[28] demonstrated that these Mabs bind to both ends of the stacked disks, which establishes that these disks are bipolar (Figure 1). The results of the bipolarity of the

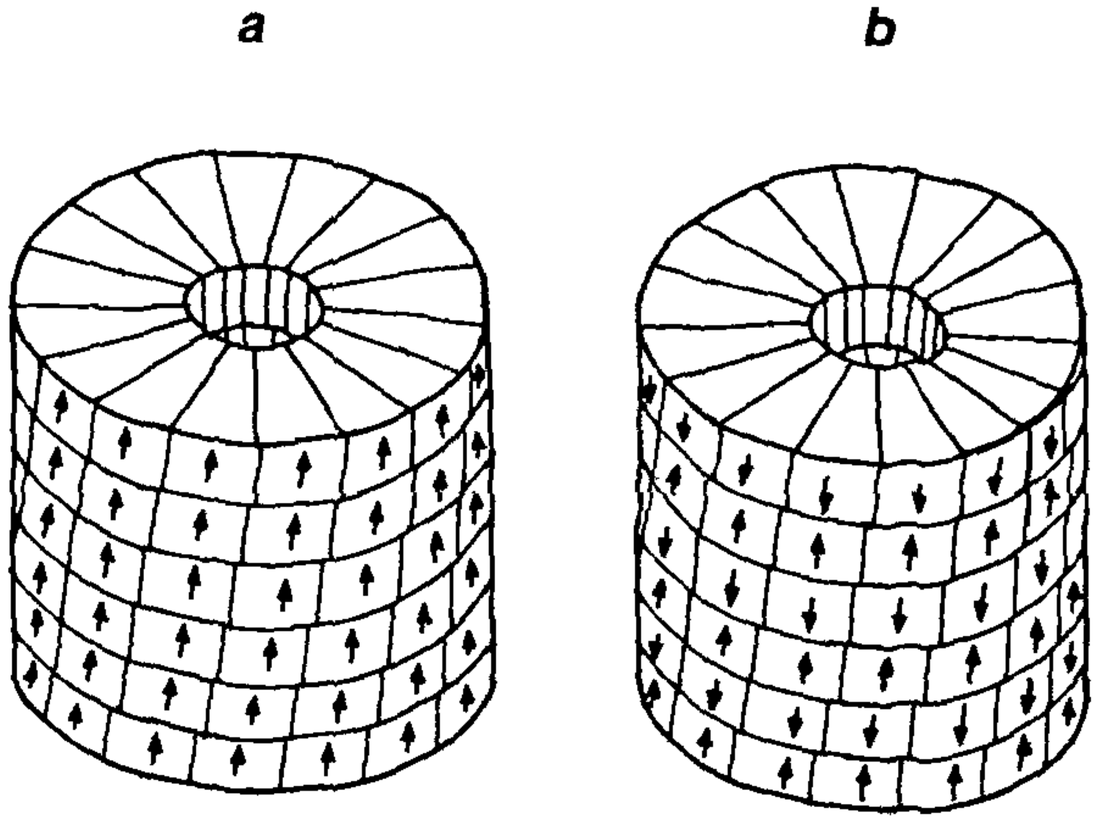

Figure 1. Topologically distinct arrangements possible for the packing of subunits in the stacked disk aggregates of TMV, *a*, polar arrangement where the two ends have distinct chemical groups, and *b*, bipolar arrangement where the two ends are structurally equivalent Monoclonal antibodies can be used to distinguish the two possible arrangements (adapted from ref 28)

stacked disk are inconsistent with the presumption that it is formed from a polar two-layer disk that also nucleates the virus assembly[32]. Since bipolar disks cannot switch to polar helices without being disassembled, it is understandable that short stacks of disks do not nucleate the virus assembly and cannot be incorporated directly into helical rods[34]. Bipolarity appears to be characteristic of all the stable disk aggregates.

TMV has a positive-sense genomic RNA that can be translated into proteins. Inside the cell, this translation begins before the uncoating of particles is complete. This prevents degradation of viral RNA by the ribonucleases and ensures efficient translation. This phenomenon is termed cotranslational disassembly[35,36]. Recently, Saunal *et al.*[37] provided evidence to show that the cotranslational disassembly of TMV could be inhibited by incubating the TMV with Mabs specific for TMV coat protein. The efficient inhibition was shown both by the antibodies that bind to the extremity of the TMV particle and the antibodies that bind to the dissociated coat protein subunits. It is possible that the efficiency of blocking of translation is dependent on the location of the epitope recognized by these antibodies.

Antigenic determinants in potyviruses

Potyviruses are economically the most important of the virus groups currently recognized[38,39]. The members of this group are characterized by long, flexuous, rod-shaped particles that are 680–900 nm long and 11 nm wide. Potyviruses have helical symmetry with a pitch of about 3.3 nm. The particles contain 5% nucleic acid and 95% protein with a sedimentation coefficient of 150S.

They consist of one copy of the positive-sense single-stranded RNA of MW $3.0–3.5 \times 10^6$ encapsidated by 2000 copies of a single protein species of molecular weight ranging from 30,000 to 37,000[38,40–45]. Complete amino acid sequence has been obtained for the coat protein of many of the potyviruses. The coat proteins from the different potyviruses vary considerably in size (263–330 amino acids), essentially because of the varying length of the N-terminus[43]. On the other hand, the C-terminal ends vary in length by only one or two residues. There are more than 180 members in this group and their classification based on biological properties such as host range, symptoms, etc., has been controversial. Serological relationships have helped to a large extent in the classification of these viruses. More recently, Mabs have been used to identify subgroups.

Mabs have been produced to several potyviruses[44–48]. Shukla *et al.*[49] examined the effect of mild trypsin treatment on six distinct potyviruses and demonstrated that both N- and C-terminal regions of the coat protein are exposed on the surface of potyvirus particles. They concluded that N-terminus is the only large region in the entire coat protein which is unique to a potyvirus and epitopes located in this region should generate virus-specific antibodies. Potyvirus group-specific epitopes are located in the trypsin-resistant core protein region and antibodies produced in this region should recognize most potyviruses (Figure 2). Shukla *et al.*[50] screened overlapping synthetic octapeptides covering the capsid protein of the Johnsongrass strain of Johnsongrass mosaic virus with five polyclonal antisera and two Mabs. This confirmed their earlier finding[49] that the epitopes

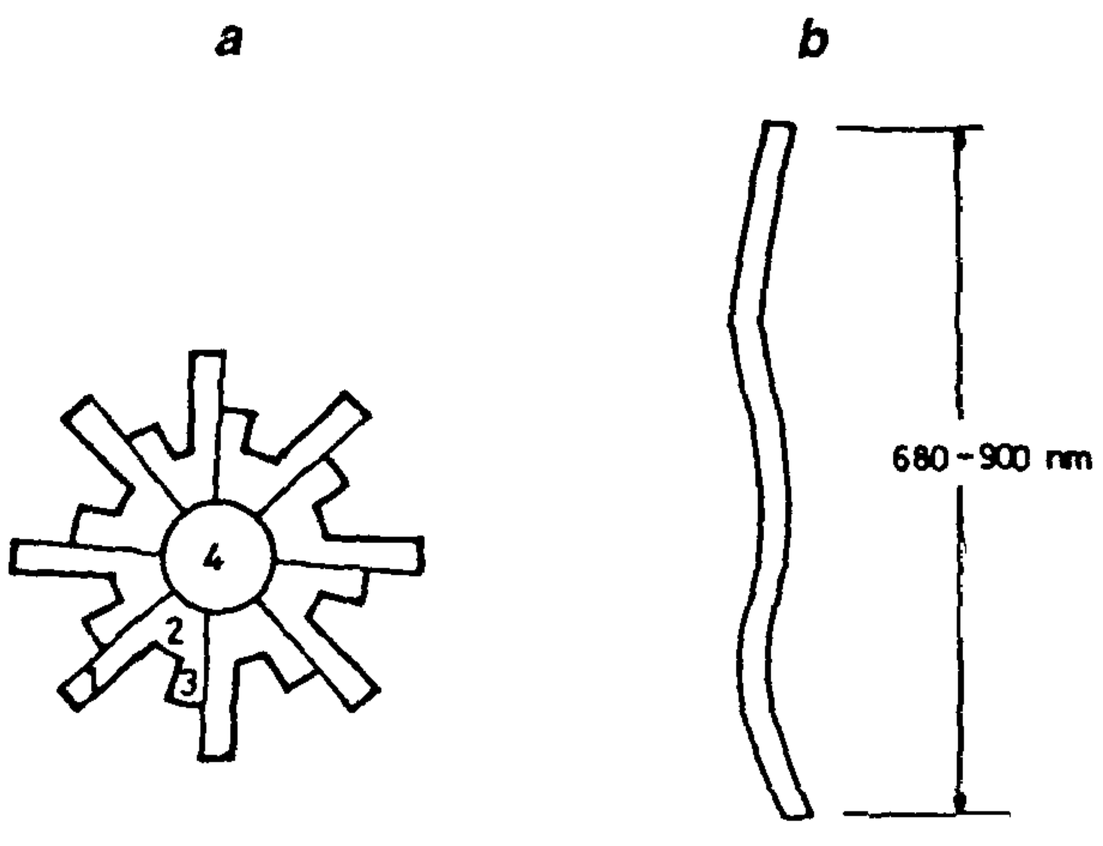

Figure 2. *a*, Schematic drawing of the 7–8 protein subunits constituting each turn of the rod-shaped potyvirus particle. The amino (1) and carboxyl termini (3) of the particles are exposed in the intact virus particles. Monoclonal antibodies to these regions are strain-specific. Monoclonals to the conserved, trypsin-resistant core (2) are potyvirus group-specific; interior of the particle which contains the genomic RNA (4) *b*, Diagrammatic representation of the flexuous, rod-shaped potyvirus particle

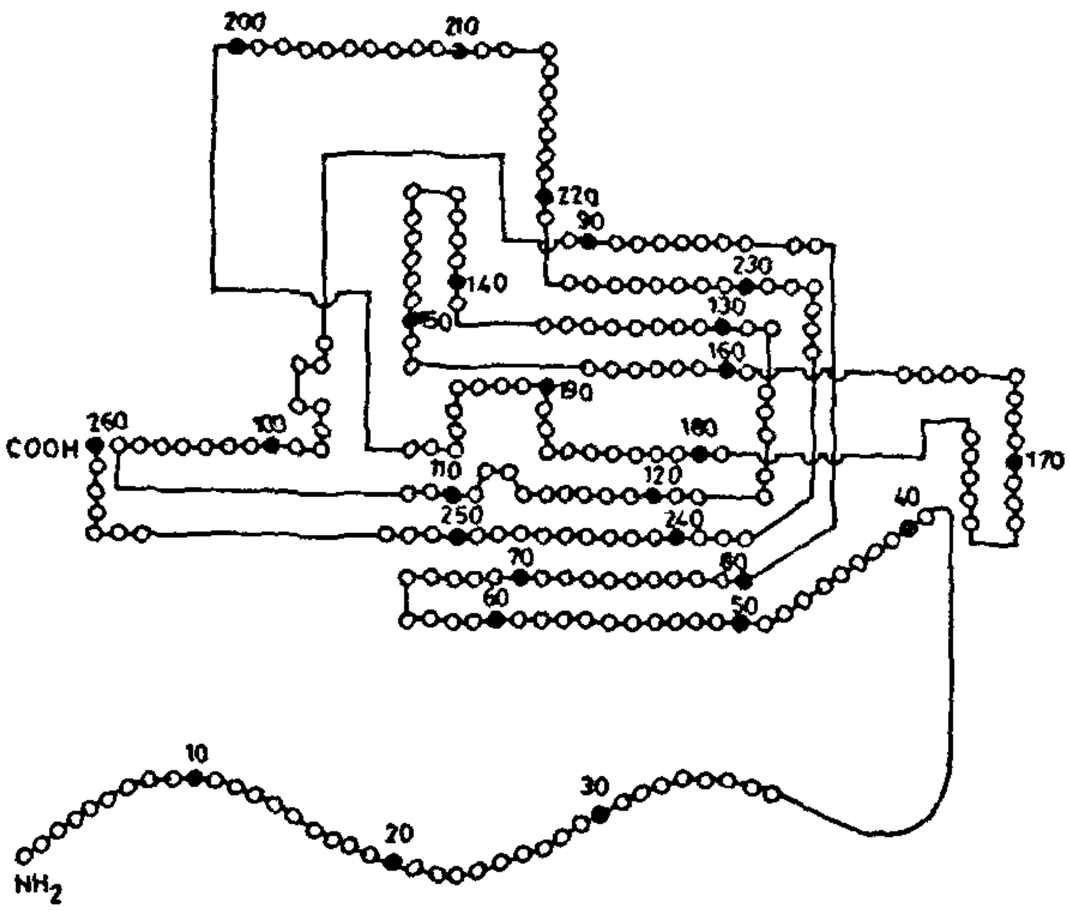

Figure 3. The order of structural domains in SBMV subunits. The R domain (residues 1–65) interacts with the RNA and is completely buried in the intact virus. Residues 65–260 constitute the surface domain (S domain) which is involved in intersubunit interactions that define the three-dimensional architecture of the capsid. Monoclonal antibodies specific to the R domain have been used to follow the disassembly pathway and hence provide information on the R-domain RNA interaction (adapted from ref. 61)

recognized by broad-spectrum cross-reactive antibodies are distributed across the entire region of the coat protein. The epitopes recognized by the virus strain-specific Mabs and polyclonal antibodies are, however, located in the N-terminal region of the coat protein. The authors did not find any antibodies that are specific to the C-terminal 18-amino acid residues, which are also exposed in the intact virus particles. However, recently, Vuento *et al.*[51], by an immunochemical analysis of overlapping synthetic hexapeptides covering the entire length of the coat protein of potato virus Y (PVY) found immunodominant regions both at the N-terminal and at the C-terminal end of the coat protein. Also, immunization of rabbits with synthetic peptides representing N- and C-terminal end of the coat protein resulted in production of antibodies that reacted with PVY. Similarly, virus-specific and cross-reactive Mabs have been raised to at least 19 different aphid-transmitted potyviruses[52]. From these studies, a panel of potyvirus broad-spectrum Mabs have been identified which can be used as reference Mabs for the detection and classification of aphid-transmitted potyviruses[52].

Use of a Mab specific for the N-terminal region of SBMV as a probe of virus structure

SBMV is a monopartite, ssRNA virus whose genome is encapsidated in a shell of 180 subunits of M_r 28 K. This

was the second plant virus structure to be determined at atomic resolution[53,54]. The viral coat protein consists of an eightstranded anti-parallel β barrel (shell domain) found in most other spherical viruses. The amino terminal arm of the protein subunit (R-domain) is rich in lysine and arginine and presumably interacts with the viral RNA and is disordered in structure (Figure 3).

Upon trypsinization, the coat protein subunits of SBMV lose the amino terminal 61 residues, resulting in a 22 K polypeptide. These subunits assemble into empty shells with a particle diameter of 20 nm, implicating the role of the N-terminal arm in the interaction with RNA and assembly of intact particles[55–60]. The positive charges in the amino terminal arm are responsible for the neutralization of most of the RNA phosphates[61].

Tremaine *et al.*[62] prepared Mabs against two strains of SBMV (SBMV-bean and SBMV-cow pea) and tested their reactivity with native virus, swollen virus (virus from which Ca^{2+} ions were removed by treatment with EDTA) and coat protein by several serological methods to gain an insight into the architecture and assembly of this virus.

One of the Mabs, 4D6, had its epitope within the first 30 amino acid residues of the N-terminal region of SBMV-c coat protein since in immunoblots 4D6 reacted with the native 29 K polypeptide but not with the 25 K proteolytic product, which lacked the N-terminal 30 amino acid residues[57,63]. The 4D6 binding site is inaccessible in the native SBMV-c particle but becomes exposed when the virus is swollen by removal of metal ions or as a result of structural changes which occur when the virus is immobilized on plastic microtitre plates. This structural perturbation which occurs on adsorption of the virus particle to a solid matrix has also been observed with other Mabs[62,64]. Attempts at collapsing the swollen virus by the addition of calcium ions or by pH adjustment did not significantly diminish the reactivity with 4D6 antibody. This indicates that the native conformation of the virus particle cannot be fully restored by these methods. The lack of recognition of native virus by 4D6 supports the earlier observation on SBMV structure and assembly that the N-terminal basic arm interacts with the RNA and is buried[57,58]. These results indicate that Mabs generated against known regions of the viral capsid protein can prove to be valuable probes of virus structure.

Antigenic structure of tymoviruses

Tymoviruses are an interesting group of spherical plant viruses which have several unique features such as formation of empty capsids *in vivo* and *in vitro*, extreme stability against digestion by ribonuclease, salt and sodium dodecyl sulphate[65]. These features suggest that intersubunit interactions dominate the stability of tymoviruses. Any attempt to disrupt the capsid structure necessarily involves harsh conditions, leading to denaturation of the coat protein. Therefore, reassembly of the virus from isolated RNA and protein has thus far not been possible. The viral coat protein also lacks the basic amino terminal arm which is known to play a role in RNA–protein interactions in several viruses[58,59,66–69]; yet, during the process of infection, the viral RNA is specifically encapsidated. This implies that there could be specific sites of RNA–protein interactions, although weak. Such types of weak RNA–protein interactions have been located by using cross-linking agents[70,71]. NMR studies on belladona mottle virus and TYMV have implicated carboxylates as probable sites of interaction with RNA[72].

TYMV, the type member of the tymovirus group of plant viruses was one of the first viruses to be crystallized[73] and its structure studied using electron microscopy and image reconstruction[74]. Subsequently, Erysimum latent tymovirus and belladona mottle tymovirus (Iowa), now renamed Physalis mottle tymovirus[75] (PhMV) were crystallized and the structure investigated by X-ray diffraction studies[76–79]. However, the high-resolution structures of these viruses are not yet available.

Immunological studies on TYMV using polyclonal antibodies demonstrated that amino acid residues 1–12, 33–45, 57–64, 143–152 and 183–189 of the coat protein are exposed in the intact virus[80–82]. On the other hand, cross-linking studies suggest that regions 1–12, 33–45 and 132–152 interact with the genomic RNA[70]. These experiments did not lead to unambiguous conclusions on the architecture of the intact virus. We have studied the architecture of PhMV using monoclonal antibodies as well as by cross-linking the RNA *in situ*. The results obtained are discussed in brief in the following section.

Architecture of PhMV

PhMV, like other tymoviruses, is an ssRNA virus whose genome is encapsidated in a protein shell made of 180 subunits of M_r 20,000. We have determined the primary structure of the coat protein of PhMV with a view to locate potential sites for protein–protein and protein–RNA interactions[83]. Further, PhMV RNA was cross-linked to its coat protein by exposure of the intact virus to ultraviolet light. The site of cross-linking of the coat protein with the RNA was identified as Lys-10 by sequencing the oligonucleotide-linked tryptic peptide obtained upon HPLC separation subsequent to enzymatic digestion of the cross-linked and dissociated virus[84]. These are the only two lysines in the N-terminal region of this protein. The identification of Lys-10 as a site for cross-linking with RNA suggests that even in this virus the weak RNA–protein interactions might be mediated via the N-terminal region of the coat protein.

Polyclonal antibodies were raised against PhMV and its denatured coat protein (PhMV-P). Analysis of the reactivity of the polyclonal antibodies with tryptic peptides of PhMV-P in dot–blot assays revealed that many of the epitopes were common to intact virus and denatured coat protein. Five Mabs to the intact virus were obtained using hybridoma technology. These Mabs reacted well with the denatured coat protein. Epitope analysis using ^{125}I-labelled Mabs suggested that probably these Mabs recognize overlapping epitopes. This was substantiated by epitope mapping using the CNBr digest of PhMV-P in western blots. All the five Mabs recognize the N-terminal 15 kDa fragment. Attempts to delineate further the specific region recognized by these Mabs by various enzymatic cleavages resulted in the loss of reactivity in all the cases. The results indicate that these Mabs probably recognize epitopes within the N-terminal 15 kDa fragment of the coat protein[85].

Three Mabs, PA3B2, PB5G9 and PF12C9, obtained using denatured coat protein as antigen, cross-reacted effectively with the intact virus indicating that the epitopes recognized by these Mabs are on the surface of the virus. Using the peptides generated by digestion with CNBr, clostripain, V-8 protease and trypsin and a recombinant protein lacking the N-terminal 21 residues expressed from a cDNA clone, it was shown that PA3B2 recognized the sequence 22–36 on the coat protein while PB5G9 and PF12C9 recognized region 75–110 (ref. 84).

These results suggest that Lys-10 is one of the specific sites through which the RNA interacts in the intact virus. The polypeptide segment (region 22–36) following this buried portion as well as the epitope within the region 75–110 are exposed in the intact virus. These observations are consistent with the canonical β-barrel structure observed in certain other plant viruses[84]. Interestingly, although hydropathy plots predicted exposed regions in the C-terminal 40 residues of PhMV-P, none of the Mabs could recognize the C-terminal 4 k CNBr fragment. Polyclonal antibodies to intact virus or denatured coat protein also did not recognize the 4k CNBr fragment. These results suggest that probably there are no sequence-specific epitopes in the C-terminal region of PhMV-P. Mabs generated using the C-terminal 4k fragment would throw light on the architecture and antigenicity of this region of the coat protein in the intact virus.

Conclusion

Mabs to viruses can serve as excellent tools towards understanding their architecture and assembly. Mabs specific to different regions of the protein could be used to identify the intermediates in the folding of coat protein and subsequently its assembly with the genome in future. In many instances, in particular in potyviruses, Mabs have also helped in identification of subgroups and their classification. More recently, the genome corresponding to variable regions of artichoke mottled crinckle virus Mab that recognizes a conserved site on the coat protein has been expressed in transgenic plants. This constitutive expression causes reduction of infection incidence and delay in symptom development[86]. Therefore, it is possible that this will be used as a more general technique to generate disease-resistant plants.

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