

interactions between the M_r 41 kDa viral Rep protein and the host factors, Rep was first over-expressed in *Escherichia coli* and its biochemical properties have been investigated. Using wheat dwarf virus (WDV) and tomato yellow leaf curl virus (TYLCV) as examples, we have demonstrated that Rep acts as a sequence specific endonuclease at the viral origin of replication. It becomes covalently linked to the 5'-end of the cleaved strand and subsequently joins single-stranded origin sequences *in vitro*. In addition, deletion analysis has shown that this nicking-joining activity maps to the amino-terminal domain of the Rep protein. Hence, the carboxy-terminal domain that contains a conserved nucleoside triphosphate (NTP) binding site is not necessary for the *in vitro* cleavage-joining activity; however, a functional NTP-binding domain is required for the full activity of the Rep protein in the replication cycle of the virus. *In vitro* phosphorylation assays have shown that cell extracts contain kinase activities that specifically phosphorylate Rep. Elucidation of the *in vivo* significance of the biochemical properties of the geminivirus Rep protein will help to unravel the molecular mechanisms involved in virus-host interactions.

Identification and characterization of wheat dwarf geminivirus from France. M Bendahmane¹, F Jouanneau¹, F de Kouchkovsky¹, H Lapiere², I Lebrun², B Gronenborn¹ (¹ CNRS, Institut des sciences végétales, F-91198 Gif-sur-Yvette; ² INRA, station de pathologie végétale, F-78026 Versailles, France)

Wheat dwarf, a prevalent disease in Northern and Eastern Europe, is transmitted by the leafhopper *Psammotettix alienus* (Dahlb). The disease 'pieds chétifs' of wheat, which has been reported in several departments of central France, is also transmitted by *P. alienus*. The typical symptoms of leaf streaking, severe stunting and reduced seed set resemble very much wheat dwarf. By molecular analyses we have identified the causative agent of 'pied chétifs' as wheat dwarf geminivirus (WDV). A novel simple and rapid method for purification of circular DNA from small amounts of plant tissue was developed and used to clone the genome of WDV-F (isolate France). We have sequenced the complete genome of WDV-F and have proven its infectivity by agroinoculation. Transmission by *P. alienus* of WDV-F from agroinoculated wheat (*Triticum aestivum* L) to healthy tester plants resulted in the

development of disease symptoms characteristic of wheat dwarf. The DNA sequence of the WDV-F genome consists of 2 750 bases and differs by only 1.3 and 1.4% from that of WDV isolates from Sweden and Czechoslovakia, respectively. A comparison of the sequence variation among the European WDV isolates with that of various isolates of maize streak virus (MSV) and other geminiviruses of Poaceae is presented. The implications for geminivirus evolution and distribution is discussed.

Transmission of geminiviruses by vectors. PG Markham (*John Innes Centre, Colney Lane, Norwich, Norfolk, NR4 7UH, UK*)

Geminiviruses are single-stranded DNA viruses with genomes of 2.5–3 kb, encapsidated in quasi-isometric particles which place them in the family *Geminiviridae*. Geminiviruses are transmitted by either whiteflies or leafhoppers (one exception is a treehopper). Discussed here are the leafhopper-transmitted geminiviruses, which infect monocotyledonous hosts, particularly maize streak virus (MSV). The actual losses caused by these viruses are difficult to establish but MSV has certainly limited production in many areas of Africa for at least the last century, and still causes epidemics. MSV is one of a complex of streak viruses that effect monocotyledons in Africa. There are a number of similar geminiviruses found in monocotyledons in Australia, Vanuatu, Japan, Asia and Europe. Indeed all these viruses may have a common origin in African grasses. All are leafhopper-transmitted and appear to have specific vector species. MSV is transmitted by several species of *Cicadulina* but the efficiency of transmission varies between species. *Cicadulina mbila* is the most efficient vector and has adapted readily to maize. In Australia the striate mosaic viruses are transmitted by different biotypes of *Nesoclutha pallida*, adapted to different hosts. The success of a virus-vector relationship depends on a number of interactions: symptoms, localisation of the virus in the plant, the feeding behaviour of the insect, the variety of plant, ecological factors, environment and other influences.

Monocot-infecting geminiviruses have a genome with 4 open reading frames, which code for replicative genes in the complementary sense (C) and a movement protein and the coat protein (CP) in the virion sense (V). In MSV point mutations in either C1 or V1 affect host range or have an effect on symptoms. The coat protein is

highly conserved and shows little variation in sequence over 12 isolates. CP is also essential for infectivity. The coat protein also determines vector specificity. The relationship with the vector is circulative and non-propagative. The virus is found in most cells of the plant except the sieve elements and can be acquired in a few seconds from infected maize by the vector. It enters the insect through the filter chamber, reaching the haemolymph, and then circulates to the salivary glands. Transmission is only effective when the insect feeds for a sustained period in the sieve elements. The success of the acquisition–transmission process is affected by factors which influence the insect–plant interactions, such as the species of host plant, the cultivar or variety and the colour of the leaves (including symptoms). The vectors and the viruses of MSV have evolved with grasses in Africa; the 2 crops most at risk in the past have been sugar-cane and maize, both introduced crops. MSV is a disease of annual grasses which is ideally suited to maize. Wheat and other small grain cereals have been affected by different strains or viruses within the streak complex but other crops such as sorghum appear resistant. *Zea perennis* and *Z. diploperennis* are resistant to MSV but cultivars and hybrids show varying susceptibility. Sweetcorns are highly susceptible to MSV but many new varieties of tropical maize show high levels of tolerance, although resistance breaking strains have been reported recently. The mutation rate of the virus is unknown but perceived adaptations may be due to mixtures of genotypes within the host. Despite the presence in Europe of *Cicadulina* species, susceptible grasses and an increasing area of maize, the ecological factors required for an MSV epidemic are unlikely to be present in the foreseeable future.

Transformation of Poaceae and transgene expression. J Fütterer, I Potrykus (*Institut for Plant Sciences, ETH Zürich, Universitätstr 2, CH 8092 Zürich, Switzerland*)

Since the first generation of fertile transgenic rice in 1989, all major cereals and many grasses have been transformed. In the beginning mainly cells or structures derived from embryogenic suspension cultures were used. Establishment of such cultures was time-consuming and very genotype-dependent and plants derived from such cultures were often sterile. More recently, calli derived from immature zygotic embryos

have been used, resulting in a higher percentage of fertile plants and also providing a less genotype-dependent source of regenerable material. The most commonly used methods of DNA delivery to cells are particle bombardment, electroporation, PEG-mediated DNA uptake and recently even infection by agrobacteria. Crucial for successful transformation was the establishment of tissue culture procedures and the development of the proper selection system for each species. At present, methods exist for all major species but in most cases, the overall transformation efficiencies are still very low and in many cases transformation is not really genotype independent. Consequently, very few transgenes besides the selectable marker gene and the β -glucuronidase have been expressed in transgenic Poaceae and data about expression of non-selectable genes are still quite scarce. Promoters of the maize ubiquitin I gene, the rice actin I gene, the maize *adh 1* gene and of the CaMV ³⁵S RNA have all been used successfully for more or less constitutive expression of transgenes. Particularly the activity of the monocot promoters depends on the presence of a monocot intron and such an intron can also strongly enhance the activity of the CaMV ³⁵S promoter. A variety of tissue-specific promoters for green tissues, seeds, and phloem have also been described. The expression levels for a given promoter construct can vary over a wide range in different transgenic plants and even in different offspring from the same transformation event or the same primary transformant. Problems with stability of gene expression in subsequent generations have also been reported.

We will present data derived from the transformation work at the ETH in Zürich to support these general conclusions.

Some aspects of *in vitro* regeneration of bread wheat. S Bernard (*INRA, Plant Breeding Station, F-63039 Clermont-Ferrand cedex, France*)

In bread wheat, like in other cereal crops, shoot regeneration is of crucial importance in the application of *in vitro* methods for plant improvement, in particular gene transfer of agronomically useful traits. However, a number of problems remain to be solved in order to make cereal transformation as efficient as with dicotyledonous crops: different culture conditions should be devised for each step of the morphogenetic process; and (ii) various defects of the regenerants are often