NEW EMBO MEMBER'S REVIEW

DNA replication and cell cycle in plants: learning from geminiviruses

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Plant cell growth and development depend on continuous cell proliferation which is restricted to small regions of the plant called meristems. Infection by geminiviruses, small DNA viruses whose replicative cycle relies on host cell factors, is excluded from those proliferating areas. Since most of the replicative factors are present, almost exclusively, in proliferating cells, geminivirus infection is believed to induce a cellular state permissive for viral DNA replication, e.g. S-phase or, at least, some specific S-phase functions. The molecular basis for this effect seems to be the interference that certain geminivirus proteins exert on the retinoblastoma-related (RBR) pathway, which analogously to that of animal cells, regulates plant cell cycle activation and G₁-S transition. In some cases, geminiviruses induce cell proliferation and abnormal growth. Mechanisms other than sequestering plant RBR probably contribute to the multiple effects of geminivirus proteins on cellular gene expression, cell growth control and cellular DNA replication. Current efforts to understand the coupling of geminivirus DNA replication to cell cycle and growth control as well as the directions in which future research is aiming are reviewed.

Keywords: cell cycle/DNA replication/geminivirus/plant/retinoblastoma

Introduction

The evolutionary trends of DNA replication and cell cycle and growth control are mechanistically well conserved among eukaryotes, from yeast to humans, from flies to plants (Stillman, 1996; Gutierrez, 1998; Huntley and Murray, 1999; Mironov *et al.*, 1999; Sherr and Roberts, 1999). Some of the key regulatory components, their activators and inhibitory cofactors, their upstream effectors and downstream targets are conserved. Furthermore, many of the building blocks are so similar that, in some cases, they can substitute functionally between different species. It is noteworthy, however, that the kind of stimuli perceived by different organisms, their response to them and the pleiotropic roles that cellular regulatory proteins play, other than those as components of the cell cycle machinery, are quite distinct in multicellular organisms at the physiological and developmental levels. An illustrative example is provided by studies of the retinoblastoma (RB) tumour suppressor pathway in different animal systems (RB and

interacting proteins; Brook *et al.*, 1996; Neufeld *et al.*, 1998; Du and Dyson, 1999; Hsieh *et al.*, 1999; Macleod, 1999).

Plants have, in addition to specific metabolic pathways, unique growth characteristics, developmental patterns and body architecture. These are consequences of several plant-specific features such as the plasticity of the plant cell, which largely contributes to its ability to dedifferentiate and regenerate, the continuous post-embryonic body remodelling, which requires a continuous proliferative potential, the frequent occurrence of endoreduplication cycles and the lack of cell migration, among others. Since the original isolation of plant cdc2 homologues (Feiler and Jacobs, 1990; John *et al.*, 1990), the past decade has witnessed significant progress in the identification of plant cell cycle regulators (see, for example, John, 1996; Verma and Gu, 1996; Gutierrez, 1998; Hesse *et al.*, 1998; Huntley and Murray, 1999; Mironov *et al.*, 1999; Wei and Deng, 1999). Comparatively, study of plant DNA replication enzymes and other regulatory proteins is scanty and still very far behind studies in other eukaryotes. Steps towards identifying cell cycle and DNA replication genes in plants are necessary in order to begin to understand their roles in plant growth and development (Clark and Schiefelbein, 1997; Cebolla *et al.*, 1999; Riou-Khamlichi *et al.*, 1999) and their differences from and similarities to animal systems.

The study of geminivirus biology, in particular their DNA replication mechanism and its connection to the host cell physiology, is a research line complementary to others based on homology or genetic screenings, functional complementation analysis and genomics. This has been exploited in recent years as (i) the geminivirus replicative cycle relies extensively on host cell factors and (ii) infection appears to have a significant impact on several cellular processes. Below, I will discuss these aspects and how these studies are providing new insights into understand the coupling of DNA replication, cell cycle and growth control in plants. Comprehensive reviews covering the molecular biology of geminiviruses have appeared recently (Bisaro, 1996; Palmer and Rybicki, 1998; Gutierrez, 1999; Hanley-Bowdoin *et al.*, 1999; Lazarowitz, 1999).

Geminivirus DNA replication follows the model of 'simple genomes' with complex virus-host interactions

A large variety of plant species are susceptible to infection by geminiviruses, a family of viruses named after their unique geminate (twinned) virion morphology. The viral genome consists of one or two small circular single-stranded DNA (ssDNA) molecules (2.6–3.0 kb). Transcription of geminivirus genes occurs bidirectionally on a

Fig. 1. Genetic organization of geminiviruses. The Geminiviridae family is composed of three genera, distinguished according to their genetic organization, plant host and insect vector: mastrevirus (maize streak virus, MSV; wheat dwarf virus, WDV; bean yellow dwarf virus, BeYDV; tobacco yellow dwarf virus, TobYDV, among others), curtovirus (beet curly top virus; BCTV, among others) and begomovirus (bean golden mosaic virus, BGMV; tomato golden mosaic virus, TGMV; African cassava mosaic virus, ACMV; squash leaf curl virus, SqLCV; tomato yellow leaf curl virus, TYLCV, among others). The genetic organization of mastreviruses (the map corresponds to WDV) is unique in that (i) they contain a large (LIR) and a small (SIR) intergenic region, with which a small DNA molecule (presumed primer) is associated in the ssDNA form, (ii) the complementary sense major transcript (c-sense, toward the left) encodes the RepA protein (Dekker *et al.*, 1991; Liu *et al.*, 1998) and, after a splicing event, first observed in WDV (Schalk *et al.*, 1989), the replication initiator protein Rep, and (iii) the virion sense transcripts (v-sense, toward the right), which may also undergo splicing (Wright *et al.*, 1997), encode the movement protein (MP), also involved in nuclear transport of viral DNA (Liu *et al.*, 1999a) and systemic infection, and the capsid protein (CP). Curtoviruses (the map corresponds to BCTV) encode, on the c-sense strand, Rep, the C2 protein, a DNA replication enhancer protein (REn) and the C4 protein, involved in symptom development and induction of cell division (Stanley and Latham, 1992; Stanley *et al.*, 1992; Latham *et al.*, 1997), and MP, the V2 protein, which may have some role in DNA replication (Hormuzdi and Bisaro, 1993), and the CP on the v-sense strand. Begomoviruses (the map corresponds to TGMV) have, apart from a few exceptions, two genomic components (A and B). The A component encodes, on the c-sense, Rep (Elmer *et al.*, 1988; Etesami *et al.*, 1991), a transcriptional activator protein (TrAP; Sunter and Bisaro, 1991; Hartitz *et al.*, 1999), REn (Elmer *et al.*, 1988) and a C4 protein, and the CP on the v-sense. The B component encodes proteins involved in intra- and intercellular viral movement (BC1 and BV1; Lazarowitz, 1999). The invariant TAATATT↓AC sequence, located in the intergenic regions, contains the initiation site (↓) of rolling circle DNA replication.

double-stranded DNA (dsDNA) template from divergent promoters located in a DNA region containing *cis*-acting signals required for viral transcription and DNA replication (Figure 1).

Geminivirus DNA replication follows a rolling circle strategy (Figure 2; Saunders *et al.*, 1991; Stenger *et al.*, 1991) which resembles that of prokaryotic ssDNA replicons (Baas and Janz, 1988; Novick, 1998). The initial stage encompasses the conversion of the ssDNA genome into a dsDNA intermediate product (Figure 2; Kammann *et al.*, 1991; Saunders *et al.*, 1992). This step, poorly understood in molecular terms, must be carried out entirely by cellular enzymes. DNA primase, α -like and δ -like DNA polymerase activities have been identified in plants (Richard *et al.*, 1991; Bryant *et al.*, 1992; Laquel *et al.*, 1993; Coello and Vazquez-Ramos, 1995a; Garcia *et al.*, 1997), but a direct demonstration of their participation in geminivirus DNA replication still awaits further

work (for a detailed discussion, see Gutierrez, 1999). This line of research is of special importance as it should help in the identification of plant genes encoding DNA polymerase and accessory factors.

Initiation of DNA replication during the second stage, the rolling circle phase, requires the concerted action of the viral Rep protein (and perhaps other viral proteins) with cellular factors, and leads to the production of new dsDNA and ssDNA viral forms (Figure 2; Stenger *et al.*, 1991; Heyraud *et al.*, 1993; Stanley, 1995). In this respect, it conforms to the so-called 'simple replicon model' (DePamphilis, 1996). The DNA replication origin has a modular architecture (Fontes *et al.*, 1994a; Sanz-Burgos and Gutierrez, 1998) and falls into two major categories: the mastrevirus-type origin, e.g. wheat dwarf virus (WDV; Kammann *et al.*, 1991; Hofer *et al.*, 1992; Schneider *et al.*, 1992; Sanz-Burgos and Gutierrez, 1998), consists of a large *cis*-acting region where the initiator Rep protein

Fig. 2. A simplified version of the geminivirus DNA replication cycle and intercellular movement of viral DNA. Geminivirus DNA replication occurs in two stages. First, the ssDNA is converted into dsDNA with the participation of cellular factors. The dsDNA serves as template for viral gene expression. Secondly, the dsDNA initiates the rolling circle phase, with the participation of viral and cellular factors, to produce new ssDNA products. These can (i) re-enter the DNA replication pool, (ii) associate with CP or (iii) be transported outside the nucleus and to the neighbouring cell, most probably through plasmodesmata, with the help of viral MPs.

forms multiple complexes (Castellano *et al.*, 1999), and the begomovirus-type origin, e.g. tobacco golden mosaic virus (TGMV; Fontes *et al.*, 1994a), containing one binding site for Rep (Fontes *et al.*, 1992, 1994b; Lazarowitz *et al.*, 1992; Orozco and Hanley-Bowdoin, 1998). Further details on initiation of geminivirus DNA replication have been reviewed recently (Gutierrez, 1999; Hanley-Bowdoin *et al.*, 1999).

After initiation, recruitment of cellular replication factors is a necessary step to complete viral DNA replication. Future efforts to identify these components should concentrate on at least two complementary approaches: the biochemical characterization of replication factors and the identification of potential interactions with one or more viral proteins. In this context, preliminary results have revealed interactions between the initiator Rep protein and cellular DNA replication proteins (A.P.Sanz-Burgos and C.Gutierrez, unpublished results). The absolute requirement for host cell DNA replication factors has forced geminiviruses to develop a number of complex interactions with the host cell which are discussed in the following paragraphs.

Geminivirus proteins and the retinoblastoma pathway: one strategy, two mechanisms?

The prokaryotic-like DNA replication strategy used by geminiviruses has adapted to a eukaryotic cellular environment and life style. A characteristic feature of multicellular organisms is that most of their cells are non-proliferating, differentiated cells in which DNA replication factors are absent or functionally inactive. Plant architecture depends on a post-embryonic morphogenetic process whereby new cells are produced continuously in the meristematic regions where cell proliferation activity is confined. Also in plants, changes in proliferating activity are associated with changes in the levels of DNA replication enzymes (Coello and Vazquez-Ramos, 1995b; Benedetto *et al.*, 1996).

It is somewhat surprising that geminivirus DNA replication seems to be excluded from the actively dividing regions of the plant, called meristems (Rushing *et al.*, 1987; Horns and Jeske, 1991; Lucy *et al.*, 1996). As a consequence, geminivirus DNA replication has been suspected for a long time to be associated somehow with a virus-induced permissive state where replication factors would be available. Consistent with this view, geminivirus dsDNA replication intermediates are up to ~20-fold more abundant in S-phase nuclei of cultured cells (Accotto *et al.*, 1993). Further support came from the observation that proliferating cell nuclear antigen (PCNA), an accessory protein of DNA polymerase δ , is undetectable in non-proliferating cells but accumulates in TGMV Rep-expressing, terminally differentiated cells (Nagar *et al.*, 1995). Expression of other S-phase gene markers, e.g. histone H2B, however, does not correlate with maize streak virus (MSV) distribution in infected tissues (Lucy *et al.*, 1996).

In animal cells, the human tumour suppressor RB protein, together with other members of the so-called 'pocket family' (p107 and p130), regulate the passage of cells through the G₁ phase and the G₁-S transit of the cell cycle by modulating the activity of the E2F-DP family of transcription factors (Weinberg, 1995; Helin, 1998; Brehm and Kouzarides, 1999). A sophisticated mechanism allows the sequential phosphorylation of RB by CDK-cyclin complexes leading to the release of RB-bound E2F-DP factors needed to activate transcription of genes required for the G₁-S transition and S-phase progression (Mittnacht, 1998; Sherr and Roberts, 1999). Animal oncoviruses are able to bypass the normal RB control pathway in G₁ by the action of one oncovirus-encoded protein whose LxCxE amino acid motif mediates binding to RB (Ludlow, 1993; Moran 1993; Vousden, 1993).

The initial finding that a geminivirus protein, e.g. WDV RepA protein, contains an LxCxE motif and interacts with human pocket proteins both in yeast and *in vitro* (Xie *et al.*, 1995) was a first clue as to which mechanism could be used by geminiviruses to induce a permissive cellular state. This observation together with the identification of plant D-type cyclins (Dahl *et al.*, 1995; Soni *et al.*, 1995), which also contain an LxCxE motif, provided strong support for the notion that a plant RB-related (RBR) pathway might exist in plants. This was confirmed by the cloning of cDNAs encoding a plant RBR protein which, as expected, is able to interact with WDV RepA (Graf

et al., 1996; Xie *et al.*, 1996). Currently, plant RBR proteins have been identified in several plant species (Ach *et al.*, 1997a; Fountain *et al.*, 1999; Nakagami *et al.*, 1999; D.Dudits, personal communication; W.Gruissem, personal communication), and their study will serve to define the role of the RBR pathway in plant cell cycle transitions, cell growth and development. The LxCxE motif is conserved in most mastrevirus RepA proteins (Xie *et al.*, 1995), where it also mediates RepA–RBR interaction (Horvath *et al.*, 1998; Liu *et al.*, 1999b). Point mutations within the LxCxE motif of RepA greatly reduce or abolish binding to pocket proteins (Xie *et al.*, 1996; Liu *et al.*, 1999b).

The mastrevirus RepA and Rep proteins have identical primary sequence in their ~200 N-terminal residues (see Figure 1). The LxCxE motif is located in the region common to both proteins. In spite of this, mastrevirus Rep protein is unable to interact with RBR (Horvath *et al.*, 1998; Liu *et al.*, 1999b). It is likely that the unique C-terminal moiety of Rep somehow hinders the LxCxE motif, as C-terminal deletions of MSV Rep (Horvath *et al.*, 1998) and WDV Rep (E.Ramirez-Parra and C.Gutierrez, unpublished) are able to interact with RBR. Quite interestingly, however, in begomoviruses, e.g. TGMV, Rep, which lacks an LxCxE motif but is otherwise highly homologous in all geminiviruses, binds to RBR (Ach *et al.*, 1997a). Residues required for TGMV Rep–RBR interaction have been mapped to a region in close proximity to the Rep oligomerization domain (L.Hanley-Bowdoin, personal communication). It should be mentioned that other plant RBR-binding proteins lacking an LxCxE motif, such as the MSI-like proteins (Ach *et al.*, 1997b) or the E2F transcription factor (Ramirez-Parra *et al.*, 1999; Sekine *et al.*, 1999), have been cloned recently. The identification of amino acids crucial for RBR binding should contribute to our understanding of the mechanisms of geminivirus RepA–RBR and Rep–RBR interactions.

Therefore, it is conceivable that interaction with a plant RBR cell cycle regulatory pathway is a strategy common to all geminiviruses, although two distinct mechanisms have evolved: one, the mastrevirus-type, which depends on the RepA–RBR interaction through an LxCxE motif and another, the begomovirus-type, relying on an LxCxE-independent Rep–RBR interaction (Figure 3). Consistent with this, studies in cultured cells have shown that: (i) WDV vectors carrying point mutations in the LxCxE motif exhibit a significant reduction in their ability to replicate (Xie *et al.*, 1995); and (ii) expression of human or plant pocket proteins strongly impairs WDV DNA replication (Xie *et al.*, 1996). Furthermore, in typical dicot-infecting geminiviruses, e.g. TGMV, point mutations that reduce or impair binding to RBR alter symptom production and tissue specificity (L.Hanley-Bowdoin, personal communication). However, recent findings indicate that the situation regarding the coupling of geminivirus replication to cell cycle control is likely to be much more complex than previously anticipated. Thus, in dicot-infecting mastreviruses, e.g. bean yellow dwarf virus (BeYDV), point mutations in the LxCxE motif of the RepA protein reduce binding to plant RBR but the virus can replicate in plants and produce wild-type symptoms (Liu *et al.*, 1999b). One possibility which still has to be examined is that mastreviruses adapted to replicate in dicots have lost the functionality of the LxCxE motif,

present in their RepA protein, and have gained a begomovirus-type of interaction between Rep and RBR that is independent of an LxCxE motif.

The complexity of geminivirus–host cell interactions in relation to the RBR pathway could be comparable with that of animal oncoviruses for which a bypass of the RB pathway is well established (Ludlow, 1993; Moran, 1993; Vousden, 1993). In papovaviruses, for example, an intricate circuitry of interactions occurs between viral proteins and cell factors during the viral replication cycle, which makes some results difficult to reconcile. Thus, polyomavirus T-ag mutants with reduced RB binding have a decreased capacity to replicate viral DNA in cultured cells, but this effect is only clearly visible after infection of growth-arrested cells (Söderbärg *et al.*, 1993) or under conditions of limited growth factor availability (Tevethia *et al.*, 1997). Furthermore, although polyomavirus large T-antigen with an intact RB-binding motif is required for immortalization, an apparent lack of correlation exists between immortalizing ability and the absolute levels of binding to RB (Pilon *et al.*, 1996). Clearly, further studies are still needed before we can fully understand in molecular terms the correlation between interference with the plant RBR pathway and geminivirus infection, not to mention the many differences that may derive from the as yet unknown plant-specific regulatory functions of RBR.

Effects of geminivirus proteins on other cellular pathways

Several lines of evidence strongly support the notion that impinging on the RBR cell cycle pathway might be just one of several mechanisms that geminiviruses may use to modify the cellular physiology (Figure 4). It should be kept in mind that interference with several cell growth-related regulatory pathways, other than the RBR pathway, has well-characterized precedents among animal oncoviruses (Eick and Hermeking, 1996; Jansen-Durr, 1996).

Recently, a group of proteins termed GRAB (for geminivirus RepA binding) has been identified by their ability to interact with WDV RepA protein (Xie *et al.*, 1999). Three aspects are relevant to the present discussion. First, GRAB proteins share their ~170 N-terminal residues with an apparently plant-specific family of proteins that contain the so-called NAC domain (Aida *et al.*, 1997). It is noteworthy that these proteins play roles as diverse as in pattern formation in embryos (Souer *et al.*, 1996), flower development (Sablowski *et al.*, 1998) and leaf senescence (John *et al.*, 1997). Secondly, residues required for interaction with GRAB proteins are located within the C-terminal domain of RepA, a region which has a significant degree of conservation in all mastrevirus RepA proteins (Xie *et al.*, 1999). Thirdly, GRAB expression inhibits WDV DNA replication in cultured cells (Xie *et al.*, 1999). The functional significance of RepA–GRAB interaction, and perhaps interaction with other NAC domain-containing proteins, still needs to be addressed in whole plants, but it reveals a potentially novel pathway for geminivirus proteins to interfere with growth- and development-related proteins (Figure 4).

While some geminiviruses are phloem restricted (Abouzid *et al.*, 1988; Sanderfoot and Lazarowitz, 1996), where they could take advantage of the meristematic

Fig. 3. Model proposed for the interference of geminivirus proteins with the retinoblastoma-related (RBR) pathway. The G₁-S transition in plants is controlled, most probably, by a pathway dependent on the retinoblastoma-related (RBR) protein which associates with the E2F-DP heterodimeric transcription factor(s). In cycling cells, phosphorylation of RBR by a CDK-cyclin complex (Nakagami *et al.*, 1999) is thought to release active E2F-DP complexes required to activate G₁-S transition and S-phase progression. S-phase-specific gene expression also occurs through other mechanisms. Geminivirus RBR-binding proteins (RepA and Rep) would bypass this control by sequestering RBR from the ternary RBR-E2F-DP complex.

Fig. 4. Impact of geminivirus proteins on different host cell pathways. Viral proteins produced from the transcriptionally active dsDNA template have effects on a number of cellular pathways, some of which are still a matter of speculation. See text for details.

activity of pre-cambial cells, others have been detected outside the vascular system, in differentiated cells (Rushing *et al.*, 1987; Nagar *et al.*, 1995; Lucy *et al.*, 1996). In this context, one attractive question is whether, as a consequence of interference with cell cycle arrest controls, geminivirus proteins actually induce dedifferentiation and re-entry in the cell cycle with a concomitant passage through S-phase and subsequent cell division. TGMV-infected cells do not seem to show a commitment to divide (Nagar *et al.*, 1995). However, infection with curtoviruses (beat curly top virus; BCTV) frequently is associated with a hyperplastic response which leads to vein swelling and leaf curling (Latham *et al.*, 1997), an effect dependent on the viral-encoded C4 protein (Stanley and Latham, 1992; Latham *et al.*, 1997). Cotton leaf curl disease also results in tissue reorganization, cell proliferation, dedifferentiation and abnormal growth in infected leaves. Recently, a novel circular ssDNA, associated with the disease and resembling a nanovirus-like DNA component, has been isolated (Mansoor *et al.*, 1999), although the significance of this component is still unknown. These observations again reveal significant differences among geminiviruses. Therefore, the identification of the cellular targets of viral proteins encoded

by abnormal growth-inducing viruses would be very helpful in understanding cell cycle regulation and its relationship to plant cell growth.

One possibility is that geminiviruses do not necessarily induce cell proliferation but, rather, a cellular state in which S-phase functions are up-regulated. This can be mediated through interference with the RBR pathway by allowing E2F-dependent activation of S-phase-specific genes. However, this might not be the only mechanism, as transcription of some S-phase-specific genes is mediated by *cis*-acting signals other than the E2F consensus sequence (Taoka *et al.*, 1999). Another way of affecting cellular gene expression could be achieved directly by geminivirus proteins, e.g. Rep, TrAP or RepA, for which a transcriptional activity has been well documented (reviewed in Lazarowitz, 1992; Bisaro, 1996; Palmer and Rybicki, 1998; Hanley-Bowdoin *et al.*, 1999).

Interestingly, changes in the nuclear architecture seem to be an early consequence of TGMV infection, and both viral and chromosomal DNA incorporate bromodeoxyuridine (cited in Hanley-Bowdoin *et al.*, 1999). If a geminivirus-dependent stimulation of semi-conservative cellular DNA replication is demonstrated conclusively, it will be crucial to determine its consequences on the infected cell

and, eventually, on the whole plant. Questions such as (i) whether an S-phase-like state is sensed as abnormal by the infected cell and is able to trigger a cell death-like process, (ii) whether a cellular defence mechanism is activated, e.g. a mechanism mediated by post-translational gene silencing (PTGS; Baulcombe, 1996; Brigneti *et al.*, 1998) and (iii) whether any viral-dependent countermeasure exists, are virtually unexplored areas. In this context, although far from being fully understood, are two recent observations. First, consistent with previous results that suggested a probable function of the African cassava mosaic virus (ACMV) TrAP protein as a suppressor of an RNA-mediated defence mechanism (Hong *et al.*, 1997), this protein is an efficient suppressor of PTGS in *Nicotiana benthamiana* (Voinnet *et al.*, 1999). Secondly, TGMV TrAP transgenic plants exhibit an enhanced susceptibility to infection by DNA and RNA viruses (D.M.Bisaro, personal communication).

Studies on geminiviruses are important to the understanding of the molecular and cellular biology of geminivirus infection as a basis for a rational design of strategies for virus control. In addition, they offer us extraordinarily powerful tools with which to approach the study of basic processes in plants and the genes controlling them. Although certainly some mechanistic aspects are shared with other eukaryotes, these studies should help to delineate some of the unique properties of plant cell growth, differentiation and body architecture. Therefore, by taking advantage of the combined approaches that are under way, we should look forward to exciting developments in the near future.

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References

- Abouzid, A.M., Barth, A. and Jeske, H. (1988) Immunogold labeling of the Abutilon mosaic virus in ultrathin sections of epoxy resin embedded leaf tissue. *J. Ultrastruct. Res.*, **99**, 39–47.
- Accotto, G.P., Mullineaux, P.M., Brown, S.C. and Marie, D. (1993) *Digitaria* streak geminivirus replicative forms are abundant in S-phase nuclei of infected cells. *Virology*, **195**, 257–259.
- Ach, R.A., Durfee, T., Miller, A.B., Taranto, P., Hanley-Bowdoin, L., Zambryski, P.C. and Gruissem, W. (1997a) *RRB1* and *RRB2* encode maize retinoblastoma-related proteins that interact with a plant D-type cyclin and geminivirus replication protein. *Mol. Cell. Biol.*, **17**, 5077–5086.
- Ach, R.A., Taranto, P. and Gruissem, W. (1997b) A conserved family of WD-40 proteins binds to the retinoblastoma protein in both plants and animals. *Plant Cell*, **9**, 1595–1606.
- Aida, M., Ishida, T., Fukaki, H., Fujisawa, H. and Tasaka, M. (1997) Genes involved in organ separation in *Arabidopsis*: an analysis of the cup-shaped cotyledon mutant. *Plant Cell*, **9**, 841–857.
- Baas, P.D. and Jansz, H.S. (1988) Single-strand DNA phage origins. *Curr. Top. Microbiol. Immunol.*, **136**, 31–69.
- Baulcombe, D.C. (1996) RNA as a target and an initiator of post-transcriptional gene silencing in transgenic plants. *Plant Mol. Biol.*, **32**, 79–88.
- Benedetto, J.P., Ech-Chaoui, R., Plissonneau, J., Laquel, P., Litvak, S. and Castroviejo, M. (1996) Changes of enzymes and factors involved in DNA synthesis during wheat embryo germination. *Plant Mol. Biol.*, **31**, 1217–1225.
- Bisaro, D.M. (1996) Geminivirus DNA replication. In DePamphilis, M.L. (ed.), *DNA Replication in Eukaryotic Cells*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 833–854.
- Brehm, A. and Kouzarides, T. (1999) Retinoblastoma protein meets chromatin. *Trends Biochem. Sci.*, **24**, 142–145.
- Brigneti, G., Voinnet, O., Li, W.X., Ji, L.H., Ding, S.W. and Baulcombe, D.C. (1998) Viral pathogenicity determinants are suppressors of transgene silencing in *Nicotiana benthamiana*. *EMBO J.*, **17**, 6739–6746.
- Brook, A., Xie, J.E., Du, W. and Dyson, N. (1996) Requirements for dE2F function in proliferating cells and in post-mitotic differentiating cells. *EMBO J.*, **15**, 3676–3683.
- Bryant, J., Fitchett, P., Hughes, S. and Sibson, D. (1992) DNA polymerase α in pea is part of a large multiprotein complex. *J. Exp. Bot.*, **43**, 31–40.
- Castellano, M.M., Sanz-Burgos, A.P. and Gutierrez, C. (1999) Initiation of DNA replication in an eukaryotic rolling-circle replicon: identification of multiple DNA–protein complexes at the geminivirus origin. *J. Mol. Biol.*, **290**, 639–652.
- Cebolla, A., Vinardell, J.M., Kiss, E., Oláh, B., Roudier, F., Kondorosi, A. and Kondorosi, E. (1999) The mitotic inhibitor ccs52 is required for endoreduplication and ploidy-dependent cell enlargement in plants. *EMBO J.*, **18**, 4476–4484.
- Clark, S.E. and Schiefelbein, J.W. (1997) Expanding insights into the role of cell proliferation in plant development. *Trends Cell Biol.*, **7**, 454–458.
- Coello, P. and Vazquez-Ramos, J. (1995a) Studies on the processivity of maize DNA polymerase 2, an α -type enzyme. *Plant Physiol.*, **109**, 645–650.
- Coello, P. and Vazquez-Ramos, J. (1995b) Maize DNA polymerase 2 is a phosphoprotein with increasing activity during germination. *Eur. J. Biochem.*, **231**, 99–103.
- Dahl, M., Meskiene, I., Bögre, L., Ha, D.T.C., Swoboda, I., Hubmann, R., Hirt, H. and Heberle-Bors, E. (1995) The D-type alfalfa cyclin gene *cycMs4* complements G1 cyclin-deficient yeast and is induced in the G₁ phase of the cell cycle. *Plant Cell*, **7**, 1847–1857.
- Dekker, E.L., Woolston, C.J., Xue, Y., Cox, B. and Mullineaux, P.M. (1991) Transcript mapping reveals different expression strategies for the bicistronic RNAs of the geminivirus wheat dwarf virus. *Nucleic Acids Res.*, **19**, 4075–4081.
- DePamphilis, M.L. (1996) Origins of DNA replication. In DePamphilis, M.L. (ed.), *DNA Replication in Eukaryotic Cells*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 45–86.
- Du, W. and Dyson, N. (1999) The role of RBF in the introduction of G₁ regulation during *Drosophila* embryogenesis. *EMBO J.*, **18**, 916–925.
- Eick, D. and Hermeking, H. (1996) Viruses as pacemakers in the evolution of defence mechanisms against cancer. *Trends Genet.*, **12**, 4–6.
- Elmer, J.S., Brand, L., Sunter, G., Gardiner, W.E., Bisaro, D.M. and Rogers, S.G. (1988) Genetic analysis of tomato golden mosaic virus. II. Requirement for the product of the highly conserved AL1 coding sequence for replication. *Nucleic Acids Res.*, **16**, 7043–7060.
- Etessami, P., Saunders, K., Watts, J. and Stanley, J. (1991) Mutational analysis of complementary-sense genes of African cassava mosaic virus DNA A. *J. Gen. Virol.*, **72**, 1005–1012.
- Feiler, H.S. and Jacobs, T.W. (1990) Cell division in higher plants: a *cdc2* gene, its 34-kDa product, and histone H1 kinase activity in pea. *Proc. Natl Acad. Sci. USA*, **87**, 5397–5401.
- Fontes, E.P.B., Luckow, V.A. and Hanley-Bowdoin, L. (1992) A geminivirus replication protein is a sequence-specific DNA binding protein. *Plant Cell*, **4**, 597–608.
- Fontes, E.P.B., Gladfelter, H.J., Schaffer, R.L., Petty, I.T.D. and Hanley-Bowdoin, L. (1994a) Geminivirus replication origins have a modular organization. *Plant Cell*, **6**, 405–416.
- Fontes, E.P.B., Eagle, P.A., Sipe, P.S., Luckow, V.A. and Hanley-Bowdoin, L. (1994b) Interaction between a geminivirus replication protein and origin DNA is essential for viral replication. *J. Biol. Chem.*, **269**, 8459–8465.
- Fountain, M.D., Murray, J.A.H. and Beck, E. (1999) Isolation and characterization of a plant retinoblastoma-related gene from a photoautotrophic cell suspension culture of *Chenopodium rubrum* L. *Plant Physiol.*, **119**, 363.
- García, E., Orjuela, D., Camacho, Y., Zuñiga, J., Plasencia, J. and Vazquez-Ramos, J. (1997) Comparison among DNA polymerases 1, 2 and 3

- from maize embryos axes. A DNA primase activity copurifies with DNA polymerase. *Plant Mol. Biol.*, **33**, 445–455.
- Grafi, G., Burnett, R.J., Helentjaris, T., Larkins, B.A., DeCaprio, J.A., Sellers, W.R. and Kaelin, W.G., Jr (1996) A maize cDNA encoding a member of the retinoblastoma protein family: involvement in endoreduplication. *Proc. Natl Acad. Sci. USA*, **93**, 8962–8967.
- Gutierrez, C. (1998) The retinoblastoma pathway in plant cell cycle and development. *Curr. Opin. Plant Biol.*, **1**, 492–497.
- Gutierrez, C. (1999) Geminivirus DNA replication. *Cell. Mol. Life Sci.*, **56**, 313–329.
- Hanley-Bowdoin, L., Settlage, S.B., Orozco, B.M., Nagar, S. and Robertson, D. (1999) Geminiviruses: models for plant DNA replication, transcription and cell cycle regulation. *Crit. Rev. Plant Sci.*, **18**, 71–106.
- Hartitz, M.D., Sunter, G. and Bisaro, D.M. (1999) The tomato golden mosaic virus transactivator (TrAP) is a single-stranded DNA and zinc-binding phosphoprotein with an acidic activation domain. *Virology*, **263**, 1–14.
- Helin, K. (1998) Regulation of cell proliferation by the E2F transcription factors. *Curr. Opin. Genet. Dev.*, **8**, 28–35.
- Hesse, M., Mayer, U. and Jürgens, G. (1998) Cytokinesis in flowering plants: cellular processes and developmental integration. *Curr. Opin. Plant Biol.*, **1**, 486–491.
- Heyraud, F., Matzeit, V., Kammann, M., Schaefer, S., Schell, J. and Gronenborn, B. (1993) Identification of the initiation sequence for viral-strand DNA synthesis of wheat dwarf virus. *EMBO J.*, **12**, 4445–4452.
- Hofer, J.M.I., Dekker, E.L., Reynolds, H.V., Woolston, C.J., Cox, B.S. and Mullineaux, P.M. (1992) Coordinate regulation of replication and virion sense gene expression in wheat dwarf virus. *Plant Cell*, **4**, 213–223.
- Hong, Y., Saunders, K. and Stanley, J. (1997) Transactivation of dianthin transgene expression by African cassava mosaic virus AC2. *Virology*, **228**, 383–387.
- Hormuzdi, S.G. and Bisaro, D.M. (1993) Genetic analysis of beet curly top virus—evidence for 3 virion sense genes involved in movement and regulation of single-stranded and double-stranded DNA levels. *Virology*, **193**, 900–909.
- Horns, T. and Jeske, H. (1991) Localization of Abutilon mosaic virus (AbMV) DNA within the leaf tissue by *in situ* hybridization. *Virology*, **181**, 580–588.
- Horvath, G.V., Pettko-Szandner, A., Nikovics, K., Bilgin, M., Boulton, M., Davies, J.W., Gutierrez, C. and Dudits, D. (1998) Prediction of functional regions of the maize streak virus replication-associated proteins by protein–protein interaction analysis. *Plant Mol. Biol.*, **38**, 699–712.
- Hsieh, J., Liu, J., Kostas, S.A., Chang, C., Sternberg, P.W. and Fire, A. (1999) The RING finger/B-box factor TAM-1 and a retinoblastoma-like protein LIN-35 modulate context-dependent gene silencing in *Caenorhabditis elegans*. *Genes Dev.*, **13**, 2958–2970.
- Huntley, R.P. and Murray, J.A.H. (1999) The plant cell cycle. *Curr. Opin. Plant Biol.*, **2**, 440–446.
- Jansen-Durr, P. (1996) How viral oncogenes make the cell cycle. *Trends Genet.*, **12**, 270–275.
- John, P.C. (1996) The plant cell cycle: conserved and unique features in mitotic control. *Prog. Cell Cycle Res.*, **2**, 59–72.
- John, P.C., Sek, F.J., Carmichael, J.P. and McCurdy, D.W. (1990) p34cdc2 homologue level, cell division, phytohormone responsiveness and cell differentiation in wheat leaves. *J. Cell Sci.*, **97**, 627–630.
- John, I., Hackett, R., Cooper, W., Drake, R., Farrell, A. and Grierson, D. (1997) Cloning and characterization of tomato leaf senescence-related cDNAs. *Plant Mol. Biol.*, **33**, 641–651.
- Kammann, M., Schalk, H.-J., Matzeit, V., Schaefer, S., Schell, J. and Gronenborn, B. (1991) DNA replication of wheat dwarf virus, a geminivirus, requires two *cis*-acting signals. *Virology*, **184**, 786–790.
- Laquel, P., Litvak, S. and Castroviejo, M. (1993) Mammalian proliferating cell nuclear antigen stimulates the processivity of two wheat embryo DNA polymerases. *Plant Physiol.*, **102**, 107–114.
- Latham, J.R., Saunders, K., Pinner, M.S. and Stanley, J. (1997) Induction of plant cell division by beet curly top virus gene C4. *Plant J.*, **11**, 1273–1283.
- Lazarowitz, S.G. (1992) Geminiviruses: genome structure and gene function. *Crit. Rev. Plant Sci.*, **11**, 327–349.
- Lazarowitz, S.G. (1999) Probing plant cell structure with viral movement proteins. *Curr. Opin. Plant Biol.*, **2**, 332–338.
- Lazarowitz, S.G., Wu, L.C., Rogers, S.G. and Elmer, J.S. (1992) Sequence-specific interaction with the viral AL1 protein identifies a geminivirus DNA replication origin. *Plant Cell*, **4**, 799–809.
- Liu, L., Davies, J.W. and Stanley, J. (1998) Mutational analysis of bean yellow dwarf virus, a geminivirus of the genus Mastrevirus that is adapted to dicotyledonous plants. *J. Gen. Virol.*, **79**, 2265–2274.
- Liu, H., Boulton, M.I., Thomas, C.L., Prior, D.A.M., Oparka, K.J. and Davies, J.W. (1999a) Maize streak virus coat protein is karyophilic and facilitates nuclear transport of viral DNA. *Mol. Plant–Microbe Interact.*, **12**, 894–900.
- Liu, L., Saunders, K., Thomas, C.L., Davies, J.W. and Stanley, J. (1999b) Bean yellow dwarf virus RepA, but not Rep, binds to maize retinoblastoma protein, and the virus tolerates mutations in the consensus binding motif. *Virology*, **256**, 270–279.
- Lucy, A.P., Boulton, M.I., Davies, J.W. and Maule, A.J. (1996) Tissue specificity of *Zea mays* infection by maize streak virus. *Mol. Plant–Microbe Interact.*, **9**, 22–31 (1996).
- Ludlow, J.W. (1993) Interactions between SV40 large-tumor antigen and the growth suppressor proteins pRB and p53. *FASEB J.*, **7**, 866–871.
- Macleod, K. (1999) pRB and E2F-1 in mouse development and tumorigenesis. *Curr. Opin. Genet. Dev.*, **9**, 31–39.
- Mansoor, S., Khan, S.H., Bashir, A., Saeed, M., Zafar, Y., Malik, K.A., Briddon, R., Stanley, J. and Markham, P.G. (1999) Identification of a novel circular single-stranded DNA associated with cotton leaf curl disease in Pakistan. *Virology*, **259**, 190–199.
- Mittnacht, S. (1998) Control of pRB phosphorylation. *Curr. Opin. Genet. Dev.*, **8**, 21–27.
- Mironov, V., de Veyler, L., van Montagu, M. and Inzé, D. (1999) Cyclin-dependent kinases and cell division in higher plants—the nexus. *Plant Cell*, **11**, 509–521.
- Moran, E. (1993) Interactions of adenoviral proteins with pRB and p53. *FASEB J.*, **7**, 880–885.
- Nagar, S., Pedersen, T.J., Carrick, K.M. and Hanley-Bowdoin, L. (1995) A geminivirus induces expression of a host DNA synthesis protein in terminally differentiated plant cells. *Plant Cell*, **7**, 705–719.
- Nakagami, H., Sekine, M., Murakami, H. and Shimmyo, A. (1999) Tobacco retinoblastoma-related protein phosphorylated by a distinct cyclin-dependent kinase complex with Cdc2/cyclin D *in vitro*. *Plant J.*, **18**, 243–252.
- Neufeld, T.P., de la Cruz, A.F., Johnston, L.A. and Edgar, B.A. (1998) Coordination of growth and cell division in the *Drosophila* wing. *Cell*, **93**, 1183–1193.
- Novick, R.P. (1998) Contrasting lifestyles of rolling-circle phages and plasmids. *Trends Biochem. Sci.*, **23**, 434–438.
- Orozco, B.M. and Hanley-Bowdoin, L. (1998) Conserved sequence and structural motifs contribute to the DNA binding and cleavage activities of a geminivirus replication protein. *J. Biol. Chem.*, **273**, 24448–24456.
- Palmer, K.E. and Rybicki, E.P. (1998) The molecular biology of Mastreviruses. *Adv. Virus Res.*, **50**, 183–234.
- Pilon, A.A., Desjardins, P., Hassell, J.A. and Mes-Masson, A.M. (1996) Functional implications of mutations within polyomavirus T antigen Rb-binding domain: effects on pRB and p107 binding *in vitro* and immortalization activity *in vivo*. *J. Virol.*, **70**, 4457–4465.
- Ramirez-Parra, E., Xie, Q., Boniotti, M.B. and Gutierrez, C. (1999) The cloning of plant E2F, a retinoblastoma-binding protein, reveals unique and conserved features with animal G₁/S regulators. *Nucleic Acids Res.*, **27**, 3527–3533.
- Richard, M.-C., Litvak, S. and Castroviejo, M. (1991) DNA polymerase B from wheat embryos: a plant delta-like polymerase? *Arch. Biochem. Biophys.*, **287**, 141–150.
- Riou-Khamlichi, C., Huntley, R., Jacquard, A. and Murray, J.A.H. (1999) Cytokinin activation of *Arabidopsis* cell division through a D-type cyclin. *Science*, **283**, 1541–1544.
- Rushing, A.E., Sunter, G., Gardiner, W.E., Dute, R.R. and Bisaro, D.M. (1987) Ultrastructural aspects of tomato golden mosaic virus infection in tobacco. *Phytopathology*, **77**, 1231–1236.
- Sablowski, R.W.M. and Meyerowitz, E.M. (1998) A homolog of *NO APICAL MERISTEM* is an immediate target of the floral homeotic genes *APETALA3/PISTILLATA*. *Cell*, **92**, 93–103.
- Sanderfoot, A.A. and Lazarowitz, S.G. (1996) Getting it together in plant virus movement: cooperative interactions between bipartite geminivirus movement proteins. *Trends Cell Biol.*, **6**, 353–358.
- Sanz-Burgos, A.P. and Gutierrez, C. (1998) Organization of the *cis*-acting element required for wheat dwarf geminivirus DNA replication and visualization of a Rep protein–DNA complex. *Virology*, **243**, 119–129.
- Saunders, K., Lucy, A. and Stanley, J. (1991) DNA forms of the geminivirus African cassava mosaic virus consistent with a rolling-circle mechanism of replication. *Nucleic Acids Res.*, **19**, 2325–2330.
- Saunders, K., Lucy, A. and Stanley, J. (1992) RNA-primed complementary-sense DNA synthesis of the geminivirus African cassava mosaic virus. *Nucleic Acids Res.*, **20**, 6311–6315.

- Schalk,H.J., Matzeit,V., Schiller,B., Schell,J. and Gronenborn,B. (1989) Wheat dwarf virus, a geminivirus of graminaceous plants needs splicing for replication. *EMBO J.*, **8**, 359–364.
- Schneider,M., Jarchow,E. and Hohn,B. (1992) Mutational analysis of the 'conserved region' of maize streak virus suggests its involvement in replication. *Plant Mol. Biol.*, **19**, 601–610.
- Sekine,M., Ito,M., Uemukai,K., Maeda,Y., Nakagami,H. and Shinmyo,A. (1999) Isolation and characterization of the E2F-like gene in plants. *FEBS Lett.*, **460**, 117–122.
- Sherr,C.J. and Roberts,J.M. (1999) CDK inhibitors: positive and negative regulators of G₁-phase progression. *Genes Dev.*, **13**, 1501–1512.
- Söderbärg,K. and Magnusson,G. (1993) Lytic functions of mutant polyomavirus large T-antigen with deletion of retinoblastoma protein-binding motif. *Virology*, **193**, 281–288.
- Soni,R., Carmichel,J.P., Shah,Z.H. and Murray,J.A.H. (1995) A family of cyclin D homologs from plants differentially controlled by growth regulators and containing the conserved retinoblastoma protein interaction motif. *Plant Cell*, **7**, 85–103.
- Souer,E., van Houwelingen,A., Kloos,D., Mol,J. and Koes,R. (1996) The No Apical Meristem gene of *Petunia* is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries. *Cell*, **85**, 159–170.
- Stanley,J. (1995) Analysis of African cassava mosaic virus recombinants suggests strand nicking occurs within the conserved nonanucleotide motif during the initiation of rolling circle DNA replication. *Virology*, **206**, 707–712.
- Stanley,J. and Latham,J.R. (1992) A symptom variant of beet curly top geminivirus produced by mutation of open reading frame C4. *Virology*, **190**, 506–509.
- Stanley,J., Latham,J.R., Pinner,M.S., Bedford,I. and Markham,P.G. (1992) Mutational analysis of the monopartite geminivirus beet curly top virus. *Virology*, **191**, 396–405.
- Stenger,D.C., Revington,G.N., Stevenson,M.C. and Bisaro,D.M. (1991) Replicational release of geminivirus genomes from tandemly repeated copies: evidence for rolling-circle replication of a plant viral DNA. *Proc. Natl Acad. Sci. USA*, **88**, 8029–8033.
- Stillman,B. (1996) Comparison of DNA replication in cells from Prokarya and Eukarya. In DePamphilis,M.L. (ed.), *DNA Replication in Eukaryotic Cells*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 435–460.
- Sunter,G. and Bisaro,D.M. (1991) Transactivation in a geminivirus: AL2 gene product is needed for coat protein expression. *Virology*, **180**, 416–419.
- Taoka,K.I., Kaya,H., Nakayama,T., Araki,T., Meshi,T. and Iwabuchi,M. (1999) Identification of three kinds of mutually related composite elements conferring S phase-specific transcriptional activation. *Plant J.*, **18**, 611–623.
- Tevethia,M.J., Lacko,H.A., Kierstead,T.D. and Thompson,D.L. (1997) Adding an Rb-binding site to an N-terminally truncated simian virus 40 T antigen restores growth to high cell density, and the T common region *in trans* provides anchorage-independent growth and rapid growth in low serum concentrations. *J. Virol.*, **71**, 1888–1896.
- Verma,D.P.S. and Gu,X. (1996) Vesicle dynamics during cell-plate formation in plants. *Trends Plant Sci.*, **1**, 145–149.
- Voinnet,O., Pinto,Y.M. and Baulcombe,D.C. (1999) Suppression of gene silencing: a general strategy used by diverse DNA and RNA viruses of plants. *Proc. Natl Acad. Sci. USA*, **96**, 14147–14152.
- Vousden,K. (1993) Interactions of human papillomavirus transforming proteins with the products of tumor suppressor genes. *FASEB J.*, **7**, 872–879.
- Wei,N. and Deng,X.W. (1999) Making sense of the COP9 signalosome. a regulatory protein complex conserved from *Arabidopsis* to human. *Trends Genet.*, **15**, 98–103.
- Weinberg,R.A. (1995) The retinoblastoma protein and the cell cycle control. *Cell*, **81**, 323–330.
- Wright,E.A., Heckel,T., Groenendijk,J., Davies,J.W. and Boulton,M.I. (1997) Splicing features in maize streak virus virion- and complementary-sense gene expression. *Plant J.*, **12**, 1285–1297.
- Xie,Q., Suárez-López,P. and Gutiérrez,C. (1995) Identification and analysis of a retinoblastoma binding motif in the replication protein of a plant DNA virus: requirement for efficient viral DNA replication. *EMBO J.*, **14**, 4073–4082.
- Xie,Q., Sanz-Burgos,A.P., Hannon,G.J. and Gutierrez,C. (1996) Plant cells contain a novel member of the retinoblastoma family of growth regulatory proteins. *EMBO J.*, **15**, 4900–4908.
- Xie,Q., Sanz-Burgos,A.P., Guo,H., García,J.A. and Gutierrez,C. (1999) GRAB proteins, novel members of the NAC domain family, isolated by their interaction with a geminivirus protein. *Plant Mol. Biol.*, **39**, 647–656.

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