Identification of the nicking tyrosine of geminivirus Rep protein

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Abstract The replication initiator (Rep) proteins of geminiviruses perform a DNA cleavage and strand transfer reaction at the viral origin of replication. As a reaction intermediate, Rep proteins become covalently linked to the 5′ end of the cleaved DNA. We have used tomato yellow leaf curl virus Rep protein for in vivo and in vitro analyses. Isolating a covalent peptide–nucleotide complex, we have identified the amino acid of Rep which mediates cleavage and links the protein to DNA. We show that tyrosine-103, located in a conserved sequence motif, initiates DNA cleavage and is the physical link between geminivirus Rep protein and its origin DNA.

Key words: Plant DNA virus; Replication initiation; Nucleotidyl transferases; Covalent protein DNA link; Rolling circle replication

1. Introduction

Geminiviruses are plant DNA viruses with a single-stranded circular DNA genome that is encapsidated in unique twinned particles [1–3]. The molecular biology of geminiviruses has been reviewed in [4], and details of their DNA replication were recently discussed [5].

Geminiviruses replicate by a rolling circle mechanism [6–8], initiated in the intergenic region (IR) at the nonanucleotide sequence TAATATTAC, universally conserved in all geminiviruses [9,10]. The protein that mediates replication initiation is the M_r 41 kDa Rep protein, the sole viral protein essential for replication [11–13]. Rep is encoded by either one (AL1, C1) or two open reading frames (C1-N and C1-C), in the latter case it is expressed via a differentially spliced mRNA [14, 15] (see Fig. 1).

Geminivirus Rep is a multifunctional protein: it is involved in transcriptional activation as well as repression of viral gene expression [16,17], and specific DNA recognition [18–22]. In addition, it has an ATP-/GTPase activity required in the replication process [23], it stimulates expression of proliferating cell nuclear antigen (PCNA) [24] and may interact with a plant homolog of the retinoblastoma protein [25].

Rep protein cleaves the geminivirus origin DNA 5′ of the penultimate nucleotide of the conserved nonamer (TAATATT/AC) [26–28]. Following cleavage, the 5′ end of the DNA remains covalently bound to Rep, and after a round of replication a Rep-catalyzed nucleotidyl transfer reaction releases a circular single-stranded virus genome.

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Proteins that undergo covalent linkage to DNA frequently do so via a tyrosine residue, as was shown for the site specific bacteriophage lambda integrase [29], topoisomerase I of *Saccharomyces cerevisiae* [30] or the RepA protein of phage ϕX174 [31]. In case of the transposon encoded $\gamma\delta$ resolvase, a serine residue forms the covalent link to the 5′ end of the DNA at the cleavage site [32].

Two tyrosines, both located in the amino-terminal domain of the protein, are conserved in geminivirus Rep proteins. The first one is part of the sequence motif -FLTY- whose function is still unknown, and the second one is the central amino acid of a sequence that has been suggested to catalyze replication initiation [33] (see Fig. 1) Here we provide biochemical proof that the tyrosine-103 of the Rep protein tomato yellow leaf curl virus (TYLCV) is the active amino acid of the cleavage/joining reaction.

2. Materials and methods

2.1. Construction of plasmids

Tyrosine-103 of TYLCV-Rep protein was mutated to phenylalanine-103 in the genome of TYLCV-S [34] as well as in pGEXC1 expressing the corresponding Rep protein [28] by PCR-based mutagenesis [35]. For this purpose, the mutagenic primer 5′-AAATCGAGCTCCGACGTCAAGTCCTT$_{2307}$TATCGAT$_{2300}$AAGGACGG-3′ was used on the templates pTY*Sst*I4 [34] and pGEXC1 to change codon TA$_{2307}$T (tyrosine-103) to TTT (phenylalanine) yielding the virus mutant TYLCV-C1$_{Y103F}$ and the expression plasmid pGEXC1$_{Y103F}$. For analytical purposes a mutation (C$_{2300}$ to T), silent for the amino acid sequence of Rep, was introduced to create a *Cla*I restriction site. The mutations are underlined and the numbers refer to the coordinates of the TYLCV-S sequence [34].

2.2. Replication assay

Wild-type TYLCV DNA and TYLCV DNA carrying the Y$_{103}$ to F$_{103}$ exchange in the C1 gene encoding Rep (TYLCV-C1$_{Y103F}$) were released from their bacterial vector by digestion with *Sst*I. Transfection of protoplasts derived from *N. tabacum* BY2 suspension culture cells [36] and analyses of the replicative viral forms were carried out as described [37,38]

2.3. Cleavage-joining reaction and Rep-binding to the 5′ cleavage-site

Wild-type GST-Rep was expressed from pGEXC1 and mutant GST-Rep$_{Y103F}$ from pGEXC1$_{Y103F}$. Expression, one-step enrichment, cleavage/joining of oligonucleotides and protein linkage assays to the 5′ cleavage-site were as described in [28]. TY11/13 (5′-CGT**ATAATATT/ACC**GGATGGCCGC-3′, 24 nt) and TY 39/1 (5′-GATCTTTTTTTT-TGGTAAAGCGGCCATCCGT**ATAATATT/A**-3′, 40 nt) TY11/12 (5′-CGT**ATAATATT/ACC**GGATGGCCG-3′, 23 nt). The site of cleavage within the conserved nonamer (in bold) is indicated by a slash.

2.4. Cleavage substrate mix

76 pmoles of oligonucleotide TY 39/0 (same sequence as TY39/1 but lacking the cleavable A at the 3′ end: 5′-GATCTTTTTTTTTGGTA-AAGCGGCCATCCGT**ATAATATT**-3′) were labelled at the 3′ end with 3.3 pmol [α-^{32}P]dATP ($\approx$400 Ci/mmol) by terminal desoxynucleotidyl transferase (BRL) [39]. To provide a sufficient amount of substrate

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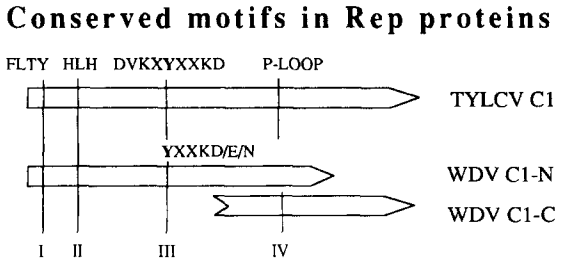

Fig. 1. Four conserved motifs (I to IV, as of [33]) of geminivirus Rep proteins are shown at their positions in the TYLCV and WDV Rep genes, respectively. The functions of motif I and II are unknown, while motif IV is part of a NTP-binding site essential for replication [23]. Motif III with the nicking tyrosine-103 differs in geminiviruses infecting dicotyledonous plants (**DVKXYXXKD**) from those infecting monocotyledonous plants (**YXXKD/E/N**). The P-loop of WDV Rep is encoded by ORF C1-C expressed from a mRNA spliced to C1-N [14,26]. TYLCV ORF C1: 359 aa, 40kDa; WDV ORF C1-N: 265 aa, 30kDa; WDV ORF C1-C: 149 aa, 17 kDa.

for cleavage, 38 pmol labelled oligonucleotide TY 39/0 were mixed with 2[illegible] nmoles unlabelled oligo TY 39/1.

2.5. Formation of a covalent GST-Rep$_{Y103}$-AMP adduct

The amino-terminal domain of Rep protein (amino acids 1–211; 23,8 kDa) was expressed in *E. coli* as a fusion to gluthatione *S*-transferase (GST; [40]) and designated GST-RepN_{24}. Construction of the expression plasmid and purification of the fusion protein was as described [25,28]. 5.8 nmol GST-RepN_{24} bound to 0.7 ml glutathione-beads were suspended in a final volume of 6 ml buffer A (25 mM Tris-HCl pH 7.5, 75 mM NaCl, 2.5 mM $MgCl_2$, 2.5 mM $MnCl_2$, 2.5 mM DTT, 0.5 mM EDTA) containing the oligonucleotide cleavage substrate mix. The reaction was stopped after 25 min at 37°C by washing with 40 ml MTPBS [40]. The glutathione-beads were equilibrated with 50 mmol NH_4HCO_3 and the bound protein was digested with 20 μg trypsin (2 h, 37°C). The extent of digestion was monitored by Laemmli-type and sequencing PAGE. Gels were stained, dried and exposed to X-ray films. After a second digestion (15 μg trypsin, 37°C, overnight) the beads were pelleted, the supernatant lyophilized, resuspended in 100 μl H_2O/0.1% TFA and applied in two aliquots to a C_{18} reversed-phase column (2.5 × 25 mm) equilibrated with H_2O/0.1% TFA. Peptides were eluted over 120 min at 0.2 ml/min with a linear 0–70% gradient (A: H_2O/0.1% TFA; B: 80% CH_3CN/0.1% TFA). Peptides were detected by absorbance at 214 nm, and fractions containing the radioactive peptide, [^{32}P]dAM, adduct were identified and quantified by their radioactivity after PAGE. The radio-labelled fractions (180 pmol) which eluted at 36% to 39% CH_3CN were combined, re-digested with 6 μg trypsin in 50 μl vol. and applied to a C_{18} reversed-phase column (2.5 × 25 mm) equilibrated with 10 mM triethylamonium acetate (pH 6.5) and eluted over 90 min with a linear 0–70% gradient (A: 10 mM triethylamonium acetate (pH 6.5); B: 70% CH_3CN/10 mM triethylamonium acetate (pH 6.5). Now, the labelled sample (60 pmol) eluted at 29% CH_3CN.

30 pmoles of this fraction were subjected to automated sequence determination by Edman degradation using a Knauer sequenator (type 810) with online phenylthiohydantoin (PTH) amino acid detection (Knauer, Berlin, Germany).

3 Results

Replication initiation by virtue of a phosphodiester bond cleavage is the function of Rep protein to which it owes its name. The reaction is initiated by the nucleophilic attack of an amino acid, often a tyrosine, and the conservation of tyrosine-103 in the sequence of TYLCV Rep protein made this the prime candidate for being involved in the cleavage [33]. Therefore, tyrosine-103 of the TYLCV Rep protein was changed to phenylalanine and the behavior of the altered Rep protein was analyzed in vivo and in vitro.

3.1. A TYLCV-C1$_{Y103F}$ mutant does not replicate

To assess the effect on replication in vivo, a single cell based replication assay was conducted. DNA of TYLCV as well as of TYLCV-C1$_{Y103F}$ was transfected into protoplasts derived from *N. tabacum* BY2 suspension cells [36]. After six days, total DNA was extracted, fractionated on an agarose gel, and hybridized in a Southern blot experiment with a TYLCV specific probe (Fig. 2a). While wild-type TYLCV produced single-stranded DNA and the double-stranded supercoiled replicative intermediate (Fig. 2a, lane 1; sc-DNA), replication of TYLCV-Rep$_{Y103F}$ was not observed (Fig. 2a, lane 2), suggesting an essential role of tyrosine-103 for TYLCV replication. In a co-transfection experiment employing equimolar amounts of TYLCV DNA and TYLCV-Rep$_{Y103F}$ DNA viral replication was not inhibited (Fig. 2a, lane 3).

3.2. Rep$_{Y103F}$ has neither DNA cleavage nor nucleotidyl transfer activity

The result of a DNA cleavage/joining assay with GST-Rep and GST-Rep$_{Y103F}$ is shown in Fig. 2b. Oligonucleotides TY11/13 (24 nt) and TY39/1 (40 nt) were labelled at their respective 5′ ends (lane 1) and equimolar amounts were incubated with either GST-Rep (lane 2) or GST-Rep$_{Y103F}$ (lane 3). Cleavage of TY11/13 yielded two products (11 nt and 13 nt) of which the one (11 nt) is visible due to its 5′ label.

The joining activity of Rep generates a 52 nt product which consists of the 5′ labelled 39 nt of TY39/1 and the transferred 13 nt of TY11/13. The alteration of tyrosine-103 into phenylalanine renders the protein inactive for both the cleavage and joining reaction (lane 3): neither the 11 nt cleavage product of TY11/13 nor the 52 nt joining product are detectable. The presence of a 40 nt/39 nt double band and of the 24 nt/23 nt (lanes 2 and 3) is due to a co-purified exonuclease activity in this particular batch of proteins. The transfer of the single 3′ terminal nucleotide of TY39/1 from Rep protein to the 3′ acceptor end of the labelled 11 nt cleavage product occurs with very low efficiency, see also [26]. Hence, the corresponding 12 nt labelled joining product was not detectable.

3.3. Rep$_{Y103F}$ is not linked to a 5′ end of DNA

To assay whether also the linkage of Rep protein to the 5′ end of cleavage site is impaired in the Rep$_{Y103F}$, oligonucleotide TY11/12 was labelled at its 3′ end. After a standard cleavage reaction with either GST-Rep or GST-Rep$_{Y103F}$, an aliquot was heat-denatured and analyzed by Laemmli-type PAGE. The gel was silver-stained and dried (Fig. 3; lanes 1 and 2). In order to detect a potential residual linkage activity a higher amount of GST-Rep$_{Y103F}$ (lane 2) in comparison to GST-Rep (lane 1) had been applied to the gel. The autoradiograph of the dried gel (lanes 1 and 2) is shown as lanes 3 and 4. Full size GST-Rep (67 kDa) became labelled due to the covalent linkage to the 3′ labelled TY11/12 cleavage product (lane 3). In addition, also GST-Rep degradation products (≈40 kDa) formed protein–DNA adducts. These products are always observed due to the instability of the fusion protein (see also [28]). In contrast, no label was found to be linked to GST-Rep$_{Y103F}$ (lane 4), despite

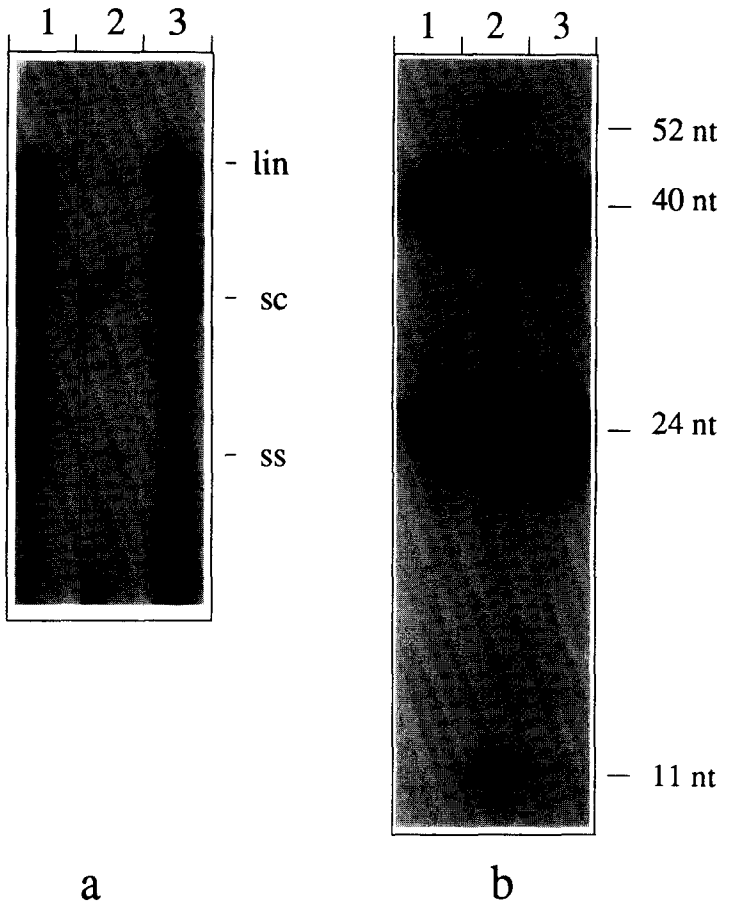

Fig. 2. (a) Replication of TYLCV and TYLCV-$C1_{Y103F}$. Protoplasts derived from *N. tabacum* BY2 suspension culture cells were transfected with cloned wild-type TYLCV DNA (lane 1), DNA of TYLCV-$C1_{Y103F}$ (lane 2) or with both DNAs in simultaneously (lane 3). Six days post-transfection, total DNA was isolated, fractionated on an 1% agarose gel and hybridized with a TYLCV specific probe in a Southern type experiment. The forms of replicative TYLCV DNA (lane 1) are indicated: lin = linear double-strand, sc = supercoiled double-strand, and ss = single-strand. No indication of viral DNA replication was detected after transfection with DNA of mutant TYLCV-$C1_{Y103F}$ (lane 2). Co-transfection of mutant viral DNA in conjugation with wild-type DNA did not inhibit DNA replication. b: in vitro DNA cleavage and joining of Rep and Rep_{Y103F}. For in vitro cleavage and joining, an equimolar mixture of 5′ end-labelled oligonucleotides TY19 (24 nt) and TY25 (40 nt) was incubated without protein (lane 1), with GST-Rep (lane 2) and GST-Rep_{Y103F} (lane 3). The reaction products were separated by a 12% PAGE. The gel which was dried and autoradiographed. The lengths indicated on the right are: 52 nt, recombinant joining product; 40 nt substrate TY25; 24 nt substrate TY19; 11 nt: cleavage product of TY19.

of the larger amount of protein analyzed, visible in the silver-stain of lane 2.

These results not only underline the general importance of tyrosine-103 for a functional Rep protein, but also strongly suggest that tyrosine-103 is required for and probably directly involved in the nicking reaction.

3.4. Identification of the amino acid that links Rep to DNA

In order to rule out any indirect influence of tyrosine-103 on the reaction, for example by a distortion of Rep protein structure, the covalent Rep-DNA adduct was isolated, and the amino acid residue linked to the DNA was identified by amino acid sequence determination.

For this purpose, we used the amino-terminal domain of Rep (GST-$RepN_{24}$) which had been shown earlier to cleave and ligate the origin of replication as efficiently as the full-size protein [26]. 5.8 nmoles of GST-$RepN_{24}$ were bound to glutathione-agarose beads, and a cleavage reaction of 23 nmoles substrate oligonucleotide mix, containing 38 pmoles of oligonucleotide TY39/0 labelled at its 3′ end (see section 2 for details) was performed with the bound protein. As a result of the reaction, a ^{32}P-labelled dAMP moiety was transferred from the 3′ end of the oligonucleotide to the active center of Rep. Subsequently, the protein was digested with trypsin, and the released peptides were separated on a C_{18} column by reversed-phase chromatography as described in section 2. 180 pmoles of labelled peptides were recovered, digested again with trypsin and separated by HPLC. Finally, 60 pmoles of a labelled peptide were recovered in a single fraction.

About 30 pmoles were subjected to automated Edman amino acid sequence determination with online PTH analysis. The identified amino acid sequence of the pure peptide was S-IDK. The yield of the identified residues corresponded to the applied quantity of peptide. The S-IDK sequence, which occurs at a single position within TYLCV Rep protein, corresponds to the sequence of a tryptic peptide comprising amino acids 102 to 106 ($SY_{103}IDK$) with tyrosine-103 at the position where no PTH amino acid signal was obtained. In the cases of DNA-linked

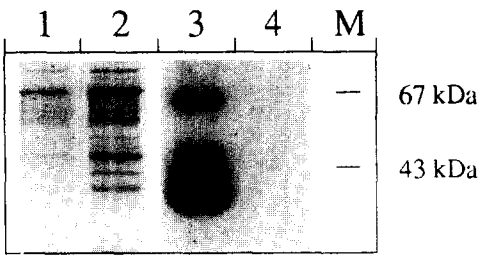

Fig. 3. The $Y_{103}F$ mutation abolishes binding of Rep to the 5′ nicksite. Oligonucleotide TY11/12 was labelled at its 3′ end and incubated with GST-Rep (lanes 1 and 3) and GST-Rep_{Y103F} (lanes 2 and 4). Subsequently, the cleavage reaction was lyophilized, resuspended in SDS loading buffer, boiled, and electrophoresed by 10% SDS-PAGE. The gel was silver-stained (lane 1 and 2), dried and autoradiographed (lane 3 and 4). Although more GST-Rep_{Y103F} than GST-Rep was loaded onto the gel (compare lane 2 with lane 1) GST-Rep_{Y103F} was not labelled (lane 4) Lanes 1 and 2 = silver stained gel; lanes 3 and 4 = autoradiograph of lane 1 and 2; M = size marker.

proteins where the active amino acid moieties were determined, the respective nucleotidyl esters also resulted in a suppression of the PTH amino acid signal [31,41,42].

The fact that only a single tryptic peptide was labelled and that the sequence of this peptide corresponds to amino acids 102–106 of Rep, with no PTH signal at position 2, unambiguously identified tyrosine-103 as the site of covalent linkage between Rep and the 5′ end of the cleaved DNA.

4. Discussion

Covalent protein–DNA links of nucleotidyl-transferases are often, but not exclusively, formed by tyrosyl-esters; also serine residues can serve as a link, as is the case for the resolvase of the transposon gamma delta [32]. We have identified tyrosine-103 of TYLC geminivirus Rep protein as the active amino acid inducing DNA cleavage and the link between the protein and the origin DNA. Since the sequence around tyrosine-103 is conserved among all geminivirus Rep proteins [33], we predict that all of them use the corresponding tyrosine to cleave the origin DNA and to form the covalent link to the DNA.

Is tyrosine-103 of Rep the sole link? The mutated Rep_{Y103F} protein did not form any covalent protein–DNA adduct, and only one labelled peptide-DNA complex could be isolated. This strongly favors the role of tyrosine-103 as the sole or predominant amino acid moiety to cleave the phosphodiester bond. It can not be completely excluded, that in a subsequent second step the DNA might be transferred from tyrosine-103 to another amino acid. However, this would have to be a very transitory liaison, otherwise we probably would have detected it, similarly as it was found for the two active tyrosine residues of the ϕX RepA protein [31].

It is obvious that during DNA replication the resolution of the covalent protein–DNA link to release a single-stranded molecule can not be mediated by a single tyrosine only; a second catalytic center in close proximity to the first one is required for the second nick of the newly synthesized origin DNA and in order to resolve the tyrosine-103–DNA bond. This second active center can be either on the same polypeptide chain, as is the case for ϕX RepA, or it may be the very same active amino acid on a second polypeptide chain, implying that active Rep protein is at least a dimer.

The question which amino acid mediates the second nucleophilic attack bears importance not only in view of the quarternary structure of active Rep, it also defines whether during rolling circle replication active Rep protein stays continuously attached to the 5′ end of the displaced strand, or whether it becomes released after completion of each replication round. Based upon tyrosine-103 being the sole identified DNA-link of Rep, two alternative mechanisms of resolution are possible.

In a mechanism requiring two active tyrosines, individual subunits of a Rep protein oligomer would provide one tyrosine each. By a nucleophilic attack of one tyrosine to the origin sequence a nick is introduced and the liberated 3′-OH serves as primer for DNA synthesis, whereas the 5′ end remains bound to Rep via the tyrosine. After one round of replication, the tyrosine of a second Rep subunit in the complex attacks the newly synthesized origin DNA and becomes itself linked to the thus created 5′ end. Concomitantly, the generated 3′-hydroxyl group serves as acceptor for the 5′ end of the DNA previously linked to the first tyrosine. As a result, a circular single-stranded DNA molecule is released, and active Rep protein remains always linked to the DNA via alternating tyrosines. Rolling circle replication of ϕX174 follows this scheme [31].

A different way of resolution would involve additional amino acids and lead to the dissociation of the Rep protein from the DNA. As in the previous mechanism, Rep first becomes linked to the 5′ end of the DNA at the cleavage site via a tyrosyl-ester. After one round of DNA synthesis, the release of a circular single-strand is mediated by the gamma carboxylate group of another amino acid. The newly synthesized origin sequence is cleaved in a nuclease type reaction, and the 5′ end, previously linked to the tyrosine, is transferred to the newly created 3′-OH end. In this case, Rep protein does not remain fixed to the new 5′ end but becomes released from the replicative intermediate. As a result, after each round of replication, Rep has to reinitiate via its active tyrosine. This type of a discontinuous replication mode was recently proposed for plasmid pC194 (see [43], to which the reader is referred for further details).

In the two alternatives, a rather different mechanism resolves the replicative intermediates and releases a circular single-strand: in the first, the tyrosine itself is the resolving nucleophil, in the second, the tyrosine and other amino acids direct the nucleophil, a water molecule. In both cases, however, the catalytic amino acids have the same backbone spacing (ϕX: **YVAKY**; pC194: **EMAKY**), a distance that is equally conserved between the tyrosine and the amino acid providing the gamma carboxylate in the exo III motif (**YXXXD**) of many polymerases. The critical importance of such a motif for the 3′–5′ exonuclease activity has been shown in detail for ϕ29 DNA polymerase [44] and *E. coli* DNA polymeraseI [45]. A close contact, i.e. hydrogen bonding of the tyrosine to the phosphodiester to be cleaved, was shown by the crystal structure of the Klenow fragment of polymerase I complexed with single-stranded DNA [46].

Also in geminivirus Rep proteins, the same backbone spacing of critical amino acids is conserved (**DVKXYXXKD** or **YXXKD/E/N**) which may suggest that in a metal assisted hydrolysis they may coordinate the divalent cation required for cleavage by both TYLCV and WDV Rep protein [26,28], and to correctly position the scissile phosphodiester bond [47].

Alternatively, D_{77} and E_{112} might be the other active site amino acids, since they represent a 'D35E motif' that executes the metal ion-dependent polynucleotidyl transferase reactions of HIV integrase [48] and bacterial transposases [49]. A strikingly similar structural organization of the catalytic centers of bacteriophage Mu A transposase and the HIV integrase domain has recently been detected [50]. Interestingly, residues D_{77} and E_{112} representing the D35E motif are also conserved in the Rep proteins of mono- and bipartite geminiviruses. Hence, the three-dimensional structure of the active center of Rep, supported by exhaustive mutagenesis data, shall ultimately clarify further details of the mechanism. In any case, its first step, the nucleophilic attack linking Rep protein to the 5′ end of the cleaved origin DNA is unequivocally shown to be mediated by tyrosine-103 of TYLCV Rep or its equivalent tyrosine in the Rep proteins of the other geminiviruses.

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References

[1] Goodman, R.M. (1977) Nature 266, 54–55.
[2] Goodman, R.M. (1977) Virology 83, 171–179.
[3] Harrison, B.D., Barker, H., Bock, K.R., Guthrie, E.J., Meredith, G. and Atkinson, M. (1977) Nature 270, 760–762.
[4] Lazarowitz, S.G. (1992) in: CRC Critical Reviews in Plant Sciences (Conger, B.V. eds.) vol. 11, pp. 327–349, CRC Press, Boca Raton.
[5] Laufs, J., Jupin, I., David, C., Schumacher, S., Heyraud-Nitschke, F. and Gronenborn, B. (1995) Biochimie (in press).
[6] Rogers, S.G., Bisaro, D.M., Fraley, R.T., Hoffmann, N.L., Brand, L., Elmer, J.S. and Lloyd, A.M. (1986) Cell 45, 593–600.
[7] Stenger, D.C., Revington, G.N., Stevenson, M.C. and Bisaro, D.M. (1991) Proc. Natl. Acad. Sci. USA 88, 8029–8033.
[8] Saunders, K., Lucy, A. and Stanley, J. (1991) Nucleic Acids Res. 19, 2325–2330.
[9] Heyraud, F., Matzeit, V., Kammann, M., Schaefer, S., Schell, J. and Gronenborn, B. (1993a) EMBO J. 12, 4445–4452.
[10] Heyraud, F., Matzeit, V., Schaefer, S., Schell, J. and Gronenborn, B. (1993b) Biochimie 75, 605–615.
[11] Elmer, J.S., Brand, L., Sunter, G., Gardiner, W., Bisaro, D.M. and Rogers, S.G. (1988) Nucleic Acids Res. 16, 7043–7060.
[12] Hanley-Bowdoin, L., Elmer, J.S. and Roger, S.G. (1990) Proc. Natl. Acad. Sci. USA 87, 1446–1450.
[13] Brough, C.L., Hayes, R.J., Morgan, A.J., Coutts, R.H.A. and Buck, K.W. (1988) J. Gen. Virol. 69, 503–514.
[14] Schalk, H.J., Matzeit, V., Schiller, B., Schell, J. and Gronenborn, B. (1989) EMBO J. 8, 359–364.
[15] Accotto, G.P., Donson, J. and Mullineaux, P.M. (1989) EMBO J. 8, 1033–1039.
[16] Hofer, J.M.I., Dekker, E.L., Reynolds, H.V., Woolston, C.J., Cox, B.S. and Mullineaux, P.M. (1992) Plant Cell 4, 213–223.
[17] Sunter, G., Hartitz, M.D. and Bisaro, D.M. (1993) Virology 195, 275–280.
[18] Fontes, E.P.B., Luckow, V.A. and Hanley-Bowdoin, L. (1992) Plant Cell 4, 597–608.
[19] Fontes, E.P.B., Eagle, P.A., Sipe, P.S., Luckow, V.A. and Hanley-Bowdoin, L. (1994) J. Biol. Chem. 269, 8459–8465.
[20] Thömmes, P., Osman, T.A.M., Hayes, R.J. and Buck, K.W. (1993) FEBS Lett. 319, 95–99.
[21] Jupin, I., Hericourt, F., Benz, B. and Gronenborn, B. (1995) FEBS Lett. 362, 116–120.
[22] Choi, I.-R. and Stenger, D.C. (1995) Virology 206, 904–912.
[23] Desbiez, C., David, C., Mettouchi, A., Laufs, J. and Gronenborn, B. (1995) Proc. Natl. Acad. Sci. USA 92, 5640–5644.
[24] Nagar, S., Pedersen, T.J., Carrick, K.M., Hanley-Bowdoin, L. and Robertson, D. (1995) Plant Cell 7, 705–719.
[25] Xie, Q., Suárez-López, P. and Gutiérrez, C. (1995) EMBO J. 14, 4073–4082.
[26] Heyraud-Nitschke, F., Schumacher, S., Laufs, J., Schaefer, S., Schell, J. and Gronenborn, B. (1995) Nucleic Acids Res. 23, 910–916.
[27] Stanley, J. (1995) Virology 206, 707–712.
[28] Laufs, J., Traut, W., Heyraud, F., Matzeit, V., Rogers, S.G., Schell, J. and Gronenborn, B. (1995) Proc. Natl. Acd. Sci. USA 92, 3879–3883.
[29] Pargellis, C.A., Nunes-Duby, S.E., Moitoso de Vargas, L. and Landy, A. (1988) J. Biol. Chem. 263, 7678–7685.
[30] Lynn, R.M., Bornsti, M.-A., Caron, P.R. and Wang, J.C. (1989) Proc. Natl. Acad. Sci. USA 86, 3559–3563.
[31] Van Mansfeld, A.D.M., Van Teeffelen, H.A.A.M., Baas, P.D. and Jansz, H.S. (1986) Nucleic Acids Res. 14, 4229–4238.
[32] Reed, R.R. and Moser, C.D. (1984) Cold Spring Harbor Symp. Quant. Biol. 49, 245–9.
[33] Ilyina, T.V. and Koonin, E.V. (1992) Nucleic Acids Res. 20, 3279–3285.
[34] Kheyr-Pour, A., Bendahmane, M., Matzeit, V., Accotto, G.P., Crespi, S. and Gronenborn, B. (1991) Nucleic Acids Res. 19, 6763–6769.
[35] Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Ehrlich, H.A. and Arnheim, N. (1985) Science 230, 1350–1354.
[36] Nagata, T., Nemoto, Y. and Hasezawa, S. (1992) Int. Rev. Cytol. 132, 1–30.
[37] Matzeit, V., Kammann, M., Schaefer, S., Schalk, H.-J., Schell, J. and Gronenborn, B. (1991) Plant Cell 3, 247–258.
[38] Maas, C. and Werr, W. (1989) Plant Cell Rep. 8, 148–151.
[39] Tu, C.-P.D. (1980) Gene 10, 177–181.
[40] Smith, D.B. and Johnson, K.S. (1988) Gene 67, 31–40.
[41] Thomas, C.D., Balson, D.F. and Shaw, W.V. (1990) J. Biol. Chem. 265, 5519–5530.
[42] Pansegrau, W., Schröder, W. and Lanka, E. (1993a) Proc. Natl. Acad. Sci. USA 90, 2925–2929.
[43] Noirot-Gros, M.-F., Bidnenko, V. and Ehrlich, S.D. (1994) EMBO J. 13, 4412–4420.
[44] Soengas, M.S., Esteban, J.A., Lazaro, J.M., Bernad, A., Blasco, M.A., Salas, M. and Blanco, L. (1992) EMBO J. 11, 4227–4237.
[45] Derbyshire, V., Grindley, N.D.F. and Joyce, C.M. (1991) EMBO J. 10, 17–24.
[46] Freemont, P.S., Friedman, J.M., Beese, L.S., Sanderson, M.R. and Steitz, T.A. (1988) Proc. Natl. Acad. Sci. USA 85, 8924–8928.
[47] Beese, L.S. and Steiz, T.A. (1991) EMBO J. 10, 25–33 .
[48] Kulkosky, J., Jones,.S., Katz, R.A., Mack, J.P.G. and Skalka, A.M. (1992) Mol. Cell. Biol. 12, 2331–2338.
[49] Fayet, O., Ramond, P., Polard, P., Prere, M.F. and Chandler, M. (1990) Mol. Microbiol. 4, 1771–1777.
[50] Rice, P. and Mizuuchi, K. (1995) Cell 82, 209–220.