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BRIEF REPORT

# Diversity and evolution of potato mop-top virus

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**Abstract** Nearly complete sequences of RNA-CP and 3′-proximal RNA-TGB were determined for 43 samples of potato mop-top virus (PMTV) originating from potato tubers and field soil from Sweden, Denmark and the USA. The results showed limited diversity and no strict geographical grouping, suggesting only a few original introductions of PMTV from the Andes. Two distinguishable types of RNA-CP and RNA-TGB were found in the samples, but no specific combination of them correlated with spraing symptoms in tubers. Lack of positive selection in the coding sequences indicates that there is no specific molecular adaptation of PMTV to new vectors or hosts.

**Keywords** Genetic variability · Phylogeny · Plant disease · *Potato mop-top virus* · Selection pressure · *Solanum tuberosum*

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The geographic origin of potato mop-top virus (PMTV; genus *Pomovirus*; family *Virgaviridae*) is probably the Andean region of South America, and it is now present in other potato-growing areas of the world, including many countries in North America, Europe and Asia [9, 22, 28]. Infection with PMTV can lead to economically important quality problems in potato production [22]. Infected tubers may show necrotic symptoms, including brown arcs and rings in the tuber flesh, and also on the surface. This spraing disease is difficult to control because PMTV is vectored by zoospores of the soil-borne plasmodiophorid *Spongospora subterranea*, and the virus can remain infective in the resting spores of the vector in soil for many years. There are no resistant potato cultivars available [22].

PMTV has tubular rod-shaped particles containing one of the three single-stranded, positive-sense RNA molecules RNA-Rep, RNA-CP and RNA-TGB. The complete genome sequence has been determined for a Swedish isolate of PMTV, with a length of 6043 nucleotides (nt) for RNA-Rep, 3134 nt for RNA-CP and 2964 nt for RNA-TGB [21, 23, 24]. RNA-Rep encodes the viral RNA-dependent RNA polymerase (RdRp) [23]. The coat protein (CP) and a larger protein (CP-RT) formed by readthrough (RT) of the CP stop codon are expressed from RNA-CP [10]. CP-RT is probably required for vector transmission [17]. RNA-TGB has four open reading frames (ORFs), where three overlapping ORFs called the triple gene block (TGB) encode three proteins (TGB1-3) essential for viral cell-to-cell

movement [19]. The fourth ORF of RNA-TGB encodes a putative cysteine-rich protein (8K protein), which appears to enhance the virulence of the virus and to act as a weak RNA silencing suppressor [13, 14].

PMTV sequences from Europe, America and Asia show a high level of identity [8, 9, 11, 15, 16, 22, 27, 28]. Compared with many other potato-infecting viruses, the genetic diversity of PMTV is low. Recent surveys have revealed a wide distribution of PMTV in the Nordic countries and the USA [2, 5, 11, 22, 27] and have made a large number of new isolates available. The aim of this study was to characterize PMTV sequences from Sweden, Denmark and the USA and to use them with sequences available from previous studies to analyse the genetic diversity and evolution of PMTV.

From a survey of PMTV distribution in Sweden [2], 20 samples from 19 counties were selected (Table 1). In addition, PMTV was baited using *Nicotiana benthamiana* and *N. debneyii* [2] from a soil sample collected in 1991 and stored at 4 °C [20]. Fourteen samples were collected from three regions of Denmark, with PMTV being obtained from soil samples using *N. benthamiana* as a bait plant and propagated by virus inoculation from infected roots to leaves [16]. In total, nine samples were obtained from five states of the USA. Samples from Idaho, Michigan, Colorado and Washington (two samples per state) consisted of symptomatic potato tubers. The sample from Oregon was obtained from roots of a bait plant of *N. tabacum* planted in field soil.

For Danish PMTV isolates, total RNA was isolated from infected test plants using an RNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's instructions. For isolates from the USA, total nucleic acids were extracted from potato tubers or bait plants [3]. Virions of Swedish isolates were captured by immunocapture using monoclonal antibodies [2]. cDNA was synthesized as described previously [2, 11, 19]. For RT-PCR, three primer pairs were used to amplify the CP-RT gene of RNA-CP as two overlapping fragments as well as the 8K gene and the 3′UTR from RNA-TGB (Fig. 1) [2, 11]. The primer CP(R) (5′-acgaattcctatgcaccagcc-3′) was used with primer CP1 [11] to amplify the CP region of USA-1, USA-3 and USA-9. Amplification was carried out using heat-stable DNA polymerases with a high level of proofreading, such as the Expand High Fidelity PCR system (Roche). PCR products for Swedish PMTV isolates were purified using a GeneJET PCR Purification Kit (Fermentas), ligated into pGEM-T Easy Vector (Promega) and introduced into Subcloning Efficiency DH5α *Escherichia coli* (Invitrogen) by transformation. Three clones from each transformation were used for sequencing at Macrogen. Sequences of amplification products for isolates from Denmark and USA were determined by direct sequencing of PCR products with the same primers that were used for PCR at MWG and Haartman Institute Sequencing Core Facility (University of Helsinki), respectively. The nucleotide sequence data reported appear in the ENA and GenBank nucleotide sequence databases (accession numbers LN614388-LN614488; Fig. 2). Pairwise identities were calculated using MegAlign (DNASTAR, Lasergene 8.0). Using MEGA 5 [26], PMTV sequences determined in this study (Table 1) and from GenBank (Supplementary Table 1) were aligned using Clustal W, and phylogenetic trees were constructed by the maximum-likelihood method with 1000 bootstrap iterations. The classification of PMTV sequences into groups was done as described previously [11]: RNA-CP-I/II (RNA2-I/II in Ref. [11]) and RNA-TGB-A/B (RNA3-A/B in Ref. [11]).

The codeml program in the PAML package version 4.6 [29] was used for likelihood ratio tests (LRTs) of positive selection on the CP gene, RT domain and 8K gene. The tests were performed separately on each gene, and only on unique sequences. We considered different models of codon evolution that allowed for variation in $\omega$ among codons but assumed the same distribution in all lineages: $\omega$ is the non-synonymous-to-synonymous rate ratio, (also known as $K_A/K_S$ or $d_n/d_s$); $\omega < 1$ indicates negative selection, $\omega = 1$ neutrality, and $\omega > 1$ positive selection. Unrooted phylogenies were constructed by the neighbor-joining method in MEGA 5 [26] and used in the codeml analyses. Three likelihood ratio tests (LRTs) were carried out as described previously [1]. To study ongoing or more recent selection, Tajima's D tests [25] in DnaSP vs 5.10 [12] were carried out on all sequences for each gene separately. A negative D value means a frequency spectrum skewed towards low-frequency alleles, whereas a positive D value can be a result of an excess of intermediate-frequency alleles in the population.

The complete coding region of the CP gene (531 nt) was characterized for 41 PMTV sequences from Sweden, Denmark and the USA (Table 1). Comparisons including 20 published PMTV sequences showed high level of nt sequence identity (98.3–100 %), and the deduced CP amino acid (aa) sequences were 96.0 to 100 % identical. Phylogenetic analysis of the CP gene (Fig. 2a) resulted in two clusters (bootstrap value, 73 %); groups CP I and CP II. No clear grouping of PMTV sequences according to geographical origin was observed.

A part of the RT domain (1617 nt, nt position 1175–2791) was characterized for 35 sequences from Sweden and Denmark (Table 1). None of them contained deletions, as were previously reported to occur in this domain [21]. Alignment with 16 published sequences revealed a high level of sequence identity, at 97.0–100 %, and a similar result was obtained for the deduced aa sequences. Phylogenetic analysis (Fig. 2b) resulted in three

**Table 1** Sequences of potato mop-top virus (PMTV) isolates determined in this study

| Sample name | Geographic origin | Source[a] | Collection year | Sequenced region |
|---|---|---|---|---|
| Sweden | | | | |
| PMTV-[SE-K] | Blekinge | Tuber | 2009 | CP-RT, 8K+3′UTR |
| PMTV-[SE-W] | Dalarna | Tuber | 2007 | CP-RT, 8K+3′UTR |
| PMTV-[SE-X] | Gävleborg | Tuber | 2008 | CP-RT, 8K+3′UTR |
| PMTV-[SE-I] | Gotland | Tuber | 2008 | CP-RT, 8K+3′UTR |
| PMTV-[SE-N-1991] | Halland | Soil | 1991 | CP-RT[b], 8K+3′UTR |
| PMTV-[SE-N] | Halland | Soil | 2006 | CP-RT, 8K+3′UTR |
| PMTV-[SE-Z] | Jämtland | Tuber | 2009 | CP-RT, 8K+3′UTR |
| PMTV-[SE-F] | Jönköping | Tuber | 2009 | CP-RT, 8K+3′UTR |
| PMTV-[SE-H] | Kalmar | Tuber | 2009 | CP-RT, 8K+3′UTR |
| PMTV-[SE-G] | Kronoberg | Tuber | 2009 | CP-RT, 8K+3′UTR |
| PMTV-[SE-T] | Örebro | Tuber | 2009 | CP-RT, 8K+3′UTR |
| PMTV-[SE-E] | Östergötland | Soil | 2006 | CP-RT, 8K+3′UTR |
| PMTV-[SE-M] | Skåne | Soil | 2006 | CP-RT, 8K+3′UTR |
| PMTV-[SE-D] | Södermanland | Tuber | 2009 | CP-RT, 8K+3′UTR |
| PMTV-[SE-AB] | Stockholm | Tuber | 2009 | CP-RT, 8K+3′UTR |
| PMTV-[SE-C] | Uppsala | Tuber | 2008 | CP-RT, 8K+3′UTR |
| PMTV-[SE-S] | Värmland | Tuber | 2008 | CP-RT, 8K+3′UTR |
| PMTV-[SE-Y] | Västernorrland | Tuber | 2008 | CP-RT, 8K+3′UTR |
| PMTV-[SE-U] | Västmanland | Tuber | 2009 | CP-RT, 8K+3′UTR |
| PMTV-[SE-O] | Västra Götaland | Tuber | 2007 | CP-RT, 8K+3′UTR |
| Denmark | | | | |
| 54-5 | Middle Jutland | Soil | 2000 | CP-RT, 8K+3′UTR |
| 54-8 | Northern Jutland | Soil | 2000 | CP-RT, 8K+3′UTR |
| 54-10 | Northern Jutland | Soil | 2000 | CP-RT |
| 54-11 | Northern Jutland | Soil | 2000 | CP-RT |
| 54-12 | Middle Jutland | Soil | 2000 | CP-RT, 8K+3′UTR |
| 54-21 | Middle Jutland | Soil | 2000 | CP-RT, 8K+3′UTR |
| 54-23 | Middle Jutland | Soil | 2000 | CP-RT, 8K+3′UTR |
| 54-24 | Funen | Soil | 2000 | CP-RT, 8K+3′UTR |
| 54-26 | Middle Jutland | Soil | 2000 | CP-RT, 8K+3′UTR |
| 54-31 | Middle Jutland | Soil | 2005 | CP-RT, 8K+3′UTR |
| 54-35 | Middle Jutland | Soil | 2005 | CP-RT, 8K+3′UTR |
| 54-42 | Middle Jutland | Soil | 2005 | CP-RT, 8K+3′UTR |
| 54-43 | Northern Jutland | Soil | 2006 | CP-RT |
| 54-44 | Middle Jutland | Soil | 2006 | CP-RT, 8K+3′UTR |
| USA | | | | |
| 1 | Idaho | Tuber | 2009 | CP, 8K |
| 2 | Idaho | Tuber | 2007 | CP, 8K |
| 3 | Michigan | Tuber | 2007 | CP, 8K |
| 4 | Michigan | Tuber | 2007 | 8K |
| 5 | Oregon | Soil | 2007 | CP, 8K |
| 7 | Colorado | Tuber | 2007 | 8K |
| 8 | Colorado | Tuber | 2007 | CP, 8K |
| 9 | Washington | Tuber | 2006 | CP |
| 10 | Washington | Tuber | 2006 | 8K |

*CP* coat protein gene, *RT* read-through domain, *8K* gene for cysteine-rich 8-kDa protein, *nt* nucleotides, *3′UTR* 3′ untranslated region

[a] Isolates were characterized directly from infected potato tubers. Isolates from the soil were acquired using bait plants

[b] Two clones were included in the analyses

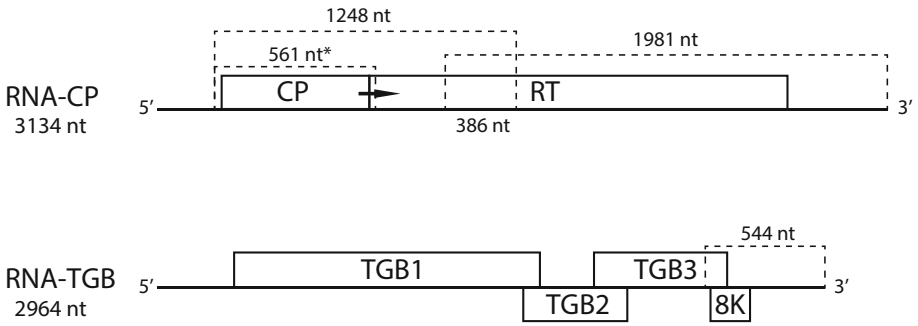


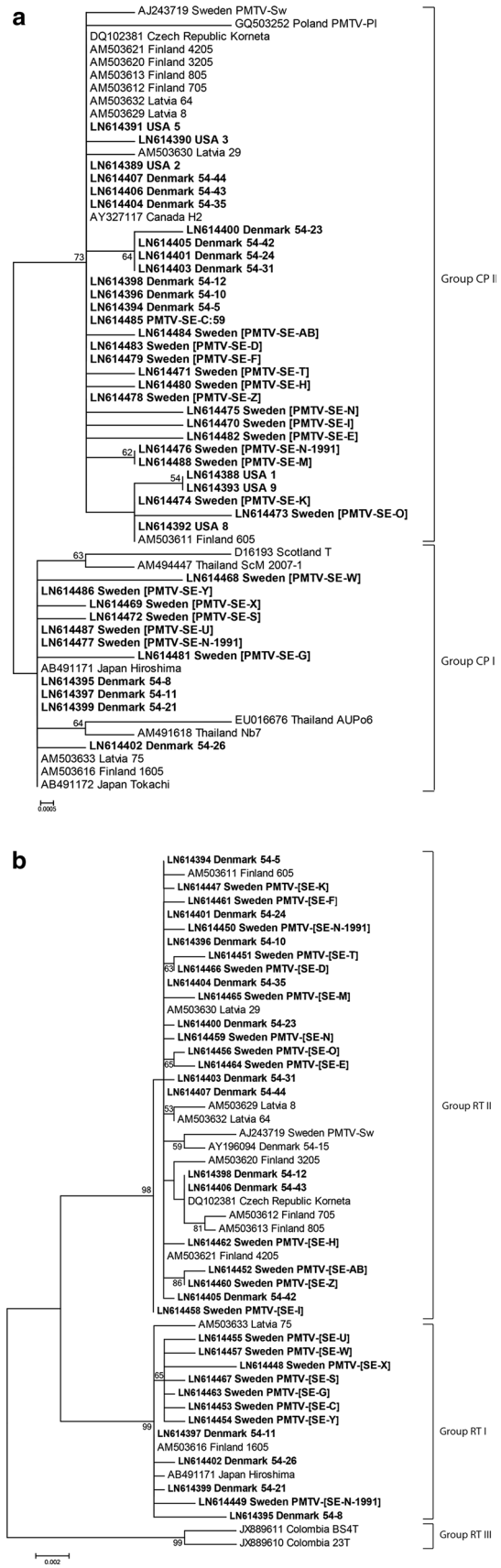

**Fig. 1** Organization of genome components RNA-CP and RNA-TGB of potato mop-top virus (PMTV). Dashed lines represent the cloned and sequenced parts of RNA-CP (two overlapping fragments of 1248 nt and 1981 nt; a fragment of 561 nt was amplified for isolates USA-1, USA-3 and USA-9) and RNA-TGB (544 nt). Genes are indicated by boxes. Translational readthrough of the stop codon is indicated by a small arrow. CP, coat protein; RT, readthrough domain; TGB1-3, triple gene block proteins; 8K, 8K protein

well-supported groups (bootstrap value, 98–99 %), with the two Colombian sequences forming one group (group RT III) and all European sequences and one from Japan located in groups RT I and II. In groups RT I and RT II, no distinct grouping could be found according to geographical origin.

The 3′-proximal part of RNA-TGB was amplified as a single RT-PCR product, including the 8K gene (207 nt) and the 3′UTR (265 nt), and 39 sequences from Sweden, Denmark and the USA were characterized (Table 1). Comparisons including published sequences revealed a high level of nt sequence identity for the 3′UTR (98.9–100 %), while the 8K gene was found to be more variable (93.3–100 % identity). Also, the deduced 8K aa sequences were variable (85.5–100 % identity), with 18 aa differences at 15 positions. A phylogenetic analysis of the 8K nt sequences gave two well-supported groups (100 % bootstrap), 8K-A and 8K-B (Fig. 2c), but without strict geographic grouping. The aa sequences within the groups 8K-A and 8K-B were very similar (98.1–100 % and 98.6–100 % identity, respectively) whereas the two groups differed at eight positions. Most differences were located in the central highly hydrophobic region of the protein [13]. The translation start codon AUG (group 8K-A) for the 8K protein was, in most sequences (8K-B), replaced by the putatively less efficient GUG [11, 13].

Sequence analysis indicated the presence of two types of RNA-CP (I and II) and RNA-TGB (A and B; Supplementary Table 2). For RNA-CP, most sequences (22/34) belonged to group II, both for the CP gene and the RT domain. The sample PMTV-[SE-C] contained the combination of RNA-CP II for the CP gene and RNA-CP I for the RT domain. Both RNA-CP I and II were found for the soil sample PMTV-[SE-N-1991]. Previously, analysis of a part of the RT domain of 21 Danish RNA-CP sequences resulted in the identification of two restrictotypes: A and B [16]. In the present study, extended sequence analysis of eight of these isolates showed the same division into two types, with RNA-CP I corresponding to type A and RNA-CP II to type B. RNA-TGB was of type B in most samples (37/39). No mixes of types A and B of RNA-TGB were found. The combination RNA-CP II/RNA-TGB B was found to be the most common (20/31), and RNA-CP I/RNA-TGB B was found in eight samples. No combination of RNA-CP or RNA-TGB was correlated with the virus sources (tuber/soil) or existence/lack of spraing symptoms. The presence of different RNA molecule combinations suggests mixed PMTV infections, which may lead to reassortment. Exchange of segments might be advantageous for the virus, but it can also lead to a loss of capability for specific interactions [6]. This may explain the predominance of a single combination of RNA-CP and RNA-TGB [11].

For the 8K gene, 68 codons were analysed, with 54 sequences included in the analysis, of which 18 sequences were unique (33 %). On average, there were 0.41 substitutions per codon, and with an overall $\omega$ ratio of 1.10, the sequences appeared to be neutrally evolving. For the CP gene, 176 codons and 59 sequences were analysed, of which 27 sequences were unique (46 %). On average, there were 0.24 substitutions per codon, and the $\omega$ ratio was 0.77, indicating that this gene was evolving under weak negative selection. For the RT gene, 538 codons and 51 sequences were analysed, of which 44 sequences were unique (86 %). On average, there were 0.26 substitutions per codon, and the $\omega$ ratio of 0.35 suggests this gene was evolving under negative selection. As expected based on the low overall $\omega$ ratios, none of the alternative models (M2a and M8) provided a significantly better fit to the data than the null models (M1a, M7 and M8a). There were 24 segregating sites among the 58 8K gene sequences, with synonymous and nonsynonymous substitutions at 9 and 15




◀**Fig. 2** Phylogenetic analysis showing predicted relationships between potato mop-top virus (PMTV) isolates based on nucleotide sequences: (**a**) complete coat protein gene (531 nt), (**b**) partial readthrough region (1617 nt), (**c**) complete 8K gene (207 nt). Sequences determined in this study are indicated in bold. Horizontal lines are in proportion to the number of nucleotide substitutions between branch nodes. Numbers represent the bootstrap values out of 1000 replicates. Only bootstrap values above 50 % are shown. Sequence accession numbers are indicated on the trees. The scale shows substitutions per site

sites, respectively. The pairwise nucleotide diversity ($\pi$) was $16.35 \times 10^{-3}$, theta ($\theta$) was $25.42 \times 10^{-3}$, and the value obtained by Tajima's D test was non-significantly negative ($D = -1.38$). Among the 59 CP gene sequences, there were 37 segregating sites, of which 13 were synonymous and 24 were nonsynonymous. $\pi$ was $4.71 \times 10^{-3}$ and $\theta$ was $15.90 \times 10^{-3}$. The value obtained by Tajima's D test was significantly negative ($D = -2.37$, $p < 0.01$), which means that there is a significant overrepresentation of low-frequency alleles for the CP gene. There were 120 segregating sites among the 51 RT domain sequences, of which 66 were synonymous and 54 nonsynonymous. $\pi$ was $8.59 \times 10^{-3}$, $\theta$ was $16.13 \times 10^{-3}$ and the value obtained by Tajima's D test was non-significantly negative ($D = -1.76$).

The results of this study confirm the previously observed limited diversity of PMTV and extend the known distribution of PMTV in the USA to the states of Oregon, Colorado and Michigan. According to the phylogenetic analysis, there is no strict geographical grouping for isolates from Europe, Asia and North America, which indicates that there have been only a few introductions from the Andes. This is in agreement with a recent study on the vector *S. subterranea* [7] in which its genetic diversity was found to be high in South America but low in other parts of the world. It has been suggested that *S. subterranea* originated in South America and was then imported with potato into Europe, from where it was subsequently disseminated to other parts of the world [7].

As found previously [11], the presence of spraing symptoms was not correlated with the type of RNA-TGB, and it is likely that all PMTV genotypes are able to induce spraing. The 8K protein sequence was highly variable, indicating a high degree of sequence flexibility. The likelihood ratio tests showed no significant positive selection for this gene, indicating only a low frequency of nonsynonymous changes. However, the 8K protein appears to be less conserved between the two distinct groups. This has also been observed for other proteins with silencing suppressor activities, such as 16K of tobacco rattle virus and p14 of beet necrotic yellow vein virus [4, 18]. Because the 8K protein acts as a virulence factor [13], the differences

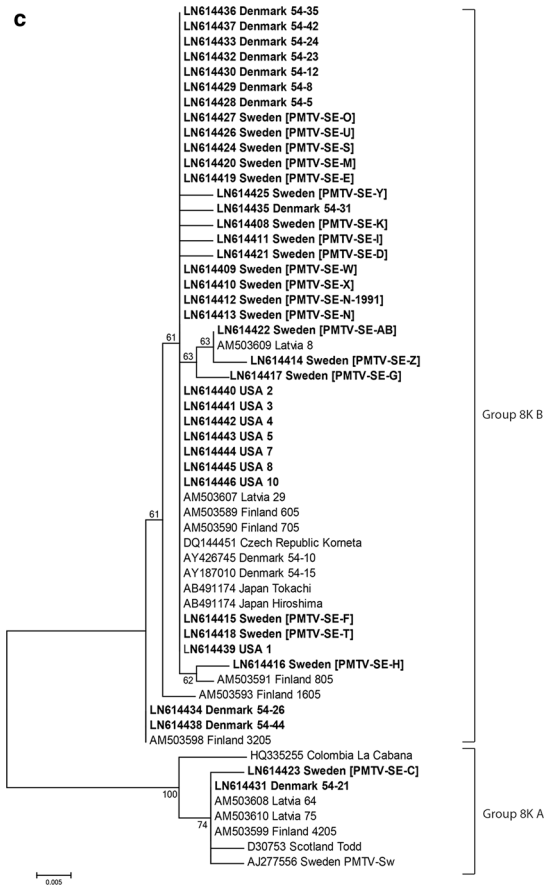



**Fig. 2** continued

may affect host adaption of PMTV isolates, which is of interest for future studies.

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