

# Divergence and conservation of the genomic RNAs of Taiwan and Hawaii strains of papaya ringspot potyvirus\*

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**Summary.** The complete nucleotide sequence of the genome of a Taiwan isolate of papaya ringspot potyvirus (PRSV YK) was determined from three overlapping cDNA clones and by direct RNA sequencing. Comparison was made with the reported Hawaii isolate of PRSV HA. Both genomes are 10326 nucleotides long, excluding the poly(A)-tail. They encode a polyprotein of 3 344 amino acids with a 5' leader of 85 nucleotides and a 3' non-translated region of 209 nucleotides. The two genomes share an overall nucleotide identity of 83.4% and an amino acid identity of 90.6%. The 3' non-translated regions show 92.3% identity. The first 23 nucleotides of the leaders are identical, while the remaining parts of the leaders only show 51.6% identity. The P1 protein genes of the two isolates are very different, with 70.9% nucleotide identity and 66.7% encoded amino acids identity. However, the other viral proteins of the two virus isolates are similar, with a 82.5-89.8% nucleotide identity of their genes and 91.2-97.6% amino acid identity, indicating that they are strains of the same potyvirus. Analysis of the ratios of nucleotide differences to the actual amino acid changes revealed that there are only 2.63 nucleotide changes for each amino acid change in the P1 protein, whereas for the other proteins 4.0-16.4 nucleotide changes are required for each amino acid replacement. The P1 protein has 58% of all the differences of polyprotein. The unusual variation in the leader sequences and the P1 proteins suggests that the two PRSV strains were derived from different evolutionary pathways in different geographic areas.

# Introduction

Papaya ringspot virus (PRSV) is a member of the *Potyvirus* genus of the family *Potyviridae* [11], with flexuous particles of  $780 \times 12$  nm and a genome consisting

\*The nucleotide sequence data reported in this paper has been deposited at EMBL under accession number X97251.

of a single stranded RNA of positive polarity [7, 33, 54]. PRSV has a single type of coat protein (CP) of 36 000 daltons (36 kDa) [16, 32] and induces both cylindrical inclusions [31] and amorphous inclusions [26] in the cytoplasm of host cells. The former consists of a 70 kDa cylindrical inclusion protein (CIP) [53] and the latter consists of a 51 kDa amorphous inclusion protein (AIP) [8].

PRSV isolates are either type P or W. Type P (papaya-infecting) isolates cause serious problems in papaya throughout the tropical and subtropical regions; while type W isolates, previously called as watermelon mosaic virus 1, are of economic importance to cucurbits throughout the world [33]. The host range of PRSV type W does not include Caricaceeae (papaya), but P isolates infect the papaya family [33, 56]. Most isolates of these two pathotypes are serologically indistinguishable when tested by the CP and CIP antisera [53, 56].

PRSV HA is an isolate originally from Hawaii and is a typical severe strain of type P [16]. This virus has been well characterized in host range [33, 56], serology [56], gene expression [54] and genetic organization [58]. In the late 1970s, PRSV spread throughout Taiwan area and severely destroyed most of the commercial papaya orchards [48]. Two major strains of PRSV type P infecting papaya were found in Taiwan, one causing mosaic symptom on papaya and the other causing wilt symptoms [5]. Wang et al. [49] reported that the mosaic strain was the most common strain in Taiwan. Although the severe Taiwan mosaic strain and Hawaii HA are distinct in pathogenicity, they cannot be differentiated by serology [56]. PRSV YK originated from Yung-Kang in Tainan area is a stable isolate with biological properties representing the most prevalent mosaic strain in Taiwan [5].

The complete nucleotide sequence and genetic organization of the RNA genome of HA has been reported [58]. The genetic organization of PRSV is similar to those of the other potyviruses except that the first protein processed from the N terminus of the polyprotein, the P1 protein, is 18 kDa to 34 kDa larger than those of all reported potyviruses. The sequence of the 2960 nucleotide residues at the 3' end of YK RNA has recently been determined and compared with those of HA, a mild strain HA 5–1, and type W strains [47], and the results indicated that the N terminus of the CP of the Taiwan YK isolate is different from those of other three strains, and this variability of the CP gene is correlated with the cross-protection specificity of the mild strain HA 5-1 [47, 49, 52, 57]. In this study, the complete nucleotide sequence of the genomic RNA of Taiwan YK isolate was elucidated and compared with that of the published Hawaii HA strain [58]. The fact that the nucleotide sequence of the genomic RNA and the amino acid sequence of the polyprotein are closely similar indicates that they are the strains of the same potyvirus. However, the greater differences in the regions of the 5' leader and the P1 protein suggest different evolutionary pathways for the derivation of the two strains.

#### Materials and methods

#### Virus purification and RNA extraction

PRSV YK, a typical mosaic strain from Taiwan, was propagated in *Cucumis metuliferus* (Naud.) Mey. [30]. The virions were purified by  $Cs_2SO_4$  centrifugation according to the method of Gonsalves and Ishii [16]. Genomic RNA was extracted by SDS-proteinase K treatment followed by sucrose density gradient centrifugation as described by Yeh and Gonsalves [54].

#### cDNA cloning, screening and in vivo excision

Complementary DNA was synthesized by the method of Gubler and Hoffman [18]. Purified PRSV YK RNA was employed as the template for cDNA synthesis, using an oligo (dT) primer with an *XhoI* site and the ZAP-cDNA synthesis kit (Stratagene, La Jolla, CA). The dsDNA was blunted by  $T_4$  DNA polymerase and then ligated with the *Eco*RI adaptor at both ends. After hydrolysis with *XhoI*, they were unidirectionally ligated to the *Eco*RI/*XhoI* site of the Uni-ZAP XR lambda phage vector (Stratagene). Recombinant phage DNAs were encapsidated with a Gigapack II XL packaging extract, plated on *E. coli* strain PLK-F' and then amplified in *E. coli* strain XL 1-Blue according to the manufacturers' instructions.

Clones corresponding to the 3' region of YK RNA were immunoscreened with the antisera to PRSV CP [56] according to the method of Huynh et al. [20]. Clones corresponding to other regions of YK RNA were selected by hybridization. Digoxigenin-labelled (Boehringer Mannheim, Germany) or <sup>32</sup>P-labelled probes nick-translated from the inserts of PRSV HA clones pCI15 [58] and pHA16 [46], which reflected the regions of nucleotide positions 3 804 to 5 334 and positions 9 062 to the poly(A)-tail of HA RNA, respectively, were used to screen the clones by hybridization.

The size of the inserts of the selected clones was determined by *Eco*RI and *Xho*I digestion of purified recombinant lambda DNA, followed by <sup>32</sup>P-end-labeling and agarose gel electrophoresis. The selected lambda recombinants were converted to phagemids by in vivo excision [37], with coinfection of a helper phage R408 or VCSM13 (Stratagene) to excise the phagemid containing the cDNA insert from the lambda DNA. Bacterial colonies containing pBluescript recombinants were recovered by plating the phagemid-infected XL 1-Blue cells on ampicillin plates. Plasmid preparation was according to the method of Davis et al. [6], and the purified plasmids were digested with *Not*I and *Kpn*I to reconfirm the size of cDNA inserts.

#### Nucleotide sequencing and direct RNA sequencing

The cDNA inserts in the lambda ZAP II vector were digested with *Not*I and *Kpn*I, and the digested fragments were subcloned in pBluescript II SK(+) or KS(+) (Stratagene) to create clones of different orientations. Subclones were selected and terminally deleted by exonuclease III/mungbean nuclease treatment [17, 19, 34]. DNA was sequenced by the dideoxynucleotide chain termination method [36], using ssDNA templates generated from the terminally deleted plasmids using the helper phage VCSM13 [37]. As the 5' end sequence of PRSV YK RNA was not obtained from the library constructed in the lambda ZAP vector, it was determined by direct RNA sequencing [59] using Moloney murine leukemia virus reverse transcriptase (Boehringer Mannheim) with the unlabeled synthetic oligonucleotide primers 5'd(GACGGTCTTTTAGTGCTATT), 5'd(CCCTCCAGGAAGGCCCTGCC),

5'd(CCGCAGTCGCATTTCTTGAA), corresponding to YK nucleotide positions 112 to 131, 278 to 297 and 422 to 441, respectively.

#### Sequence analysis

Sequence data were assembled and analyzed by the PC/Gene software (IntelliGenetics, Inc., Mountain View, CA), the MicroGenie software (Beckman Instrument, Palo Alto, CA) and the GCG package (Version 7-UNIX, Genetics Computer Group Inc., Madison, WI) [9, 10]. The complete nucleotide sequence of YK was compared with the published nucleotide sequence of PRSV HA [58]. GAPSHOW program was used to display an alignment of nucleotide or amino acid sequence by making a graph that shows the distribution of similarity and differences [9, 10].

#### **Results and discussion**

# Complete nucleotide sequence of YK RNA

Three cDNA clones, pYKD9, pYKW63 and pYKW40, were selected from the lambda ZAP library by immunoscreening or hybridization, and they contained inserts of 2.2 kb, 6.3 kb and 4.0 kb, respectively. These three clones represented 99.7% of the PRSV YK genome. Sequence data were obtained from both orientations of the subclones and from the orderly deleted clones. The 34 nucleotide residues at the 5' terminus were absent from the clone pYKW40, they were therefore determined by direct sequencing of the RNA using the oligonucleotide primers described in Materials and methods.

# Comparison of the primary structures of the genomic RNAs of Taiwan and Hawaii strains

The assembled PRSV YK RNA sequence contained 10 326 nucleotides, excluding the poly(A) tail, and the length was identical to the published PRSV HA RNA (Fig. 1). Computer translation of the YK RNA revealed a single large open reading frame (ORF) that starts at the first AUG at the nucleotide position 86 and terminates with a UGA codon at position 10118, followed by a 3' nontranslated region (NTR) of 209 nucleotides. The large ORF of YK encodes a polyprotein of 3 344 amino acids with a calculated molecular weight of 380 629 (381 kDa) (Fig. 2). The base composition of YK RNA is 31.4% adenine, 23.5% guanine, 18.4% cytosine and 26.6% uracil, which is similar to that of HA (31.2% adenine, 23.8% guanine, 18.0% cytosine and 27.0% uracil) and also similar to those of the other potyviruses [58].

Comparison of the complete nucleotide sequence of YK with that of the published Hawaii strain HA [58] indicated that they share an overall 83.4% nucleotide identity and a 90.6% amino acid identity for the polyproteins. A total of 1711 nucleotide residues was found to be different between RNAs of YK and HA, resulting in 315 amino acid changes in the polyprotein (Figs. 1 and 2).

The 5' leader sequence of YK RNA is 85 nucleotides long and has a significantly different base composition (43.5% A, 23.5% C, 5.9% G, 27.1% U) from that of the complete sequence. This is similar to the leader sequences of several

AAATAAAACATCTCAACACAAATCCAAAGCATTCAAACACACTCGAGCAAACTTTAAATTTTAAAATCTTGTCATTTGCAATCATGTCTTCATTATACCAATTGCAACCAATAGCACT T CA T C T C A ATC ATT T C C T C T G CA A CAA CA \_\_\_\_ T G ACTC T G G GC T A 120 GATTCTGCAGGCCGTTCAAAATTGGAAACACTGAAGTTGGCAGGGGCCTTCCTGGAGGGCAATAGGAGAATTGGAGCATTTTTGAAAATTGTTAAGAAAACAATGGTCGGCCGCCTGGG A C AT G G G T A A A T C ATTTG T GA T A G A CCG C T T T 360 480 600 AGCACCTGAGGAGCTGAGATTATGGAGACTAGGGGGTCTAGTGGAGGAGGTCTTCTGGAGGAGCACGGAGAATGGTGCCTATAACTACCAAAGCTGAAGAAGCATGGAGGATACA G A T A T CA G AC A TT T G GG A A AA T G TA CG T A 720 840 AAGTACAGTTCCTCAAAGTGAGATAGAGGAAGCAGCGTACCTGGCACTCGAAGTCGGTAATGAAATTGCTGAGAAGAAACCTGAACTCAAACTAACACCATATTGGAGTGCTAGTCTTGA AGGA A C T GGAA G GA A T C T C G G G T 960 1080 1200 Anticategranatticategranactegranactegrantertranstegranacanatticategranater and the second structure of the term of te 1320 GCCCTTAACACACCGCCTCAAGTGGTCTCATTTTTTAGACCAAAATTTGCTGACAGAATAGGTCGAATATTTTGGTGAATATTTCATCACGCGTGGGCGTTACGCAGGGAAATTATTTGATGG AAATC G GTT T T G C AG G G C ACG G G C C C G TGTT A A CTT G T G 1680  $\begin{array}{cccc} \text{Argate consistency of the second second$ T A A A G CTATEGO A I  $L_{2}$  RC-FO protein graceActricatariagatraceateriagatraceateriagatraceactricatariagatraceatrac  $\begin{array}{ccccc} \texttt{AATTGACGAAGTTGGTGAGGACTTGAATTGTGGACGTTTACGTGAAACTTCTCGTCATATGGGGGCTCATTGGGTCATGTTTCAACATTACTCGACCAACTGAACAGAGTTTT & 2040 \\ \texttt{T} & \texttt{G} & \texttt{T} & \texttt{AG} & \texttt{G} & \texttt{A} & \texttt{C} & \texttt{T} & \texttt{T} & \texttt{T} & \texttt{A} \\ \end{array}$  $\begin{array}{c} \texttt{TGGCAAAGCTCAACCCAGCTCTTAGCGAGCAACCAATGGACAACAATGGCAACTTCTTATGGGGTGAAAGACAATATCACGCCAAAAGATTCTTTGCAACTACTTTGAAAA 2400 \\ \texttt{T} & \texttt{T} & \texttt{G} & \texttt{C} & \texttt{T} \end{array} \end{array}$ GATTGATCACAGTAAGGGTTATGAGTACTATAGTCAAAGCCCAAAATGGCATTGGCAATGGTACTTGGCACAGTTTGGCACAGAAATTTGGAGAGGTTTGGACAGGCAAAT GAT CA G AA T CA G AA ATTGAAAACTCCCACCAAGAATCACATTGTTCTTGGTAATTCTGGTGATCCTAAGTATGTTGACTTGCCGACTCTTGAGTCTGATTGAATGTACATAGCTAAGAAAGGTTATGTTACAT GAACATCTTTCTGGCGATGCTCATAAACATACCTGAGAATGAGGCAAAGGACTTTACGAAGAGAGTTCGCGCATCTAGTAGGTTCGAAGCTTGGGGAGTGGCCAACGATGCTAGATGTCGC 2880 T T A T A T A C C A A G T C T G A A TTCTGGATATCATATACTGAAGGCTAACACAGTTGATCCAGTTGATCCAGTTCGCCAGAGAACCGCTCGATAGTGAAAACACTACATTGTCGGGTGGAGAATTTGATCCAACCACTAG 3120 T A T C C A T G C A G C G C G T A TAATAGTGGTGCAATGGAGCATGGATTGAATTATTGGATTAAAAGGGACCAAGATGTTGTGAGGTTATTGTTTAGTGAGGGAATTATGTGGCGAAAGTGACGCCTTGCTAGAACAATCCT 3360 C G T C G C A T C G C A G GCAGTTGACGGATGAAGGCCTGCTCAAGCAGGGAGTTTTCAACACTGGATCCTAGACTGCGGTGAGGGCTGTGGAAAAAACCTACGCCGCGCTCTCTGGCAGGAAGAGTGGCGTGGCGTGGCTTTAAGTTT 3600 T A A T A GT A A T A G GTTTCAAAAATGGCACTTAAGGTACTTTGGCATTCAAATCACAACCACTTTTTTCCGAGTATTTAAAGCCAAAAGGGCGCGCAGAATTGAAAATTGGTACGACTTCTCACCGAAGTACTG 3720 AAGACTCAAAGATTAAAGTTTATAGCTTGATTCACATACCAGTAATTCATCAAGCAAAAATCAGACAAAGGAAAACTCGAACAAGGAAATTGCATTCATCACATTAATTCTAATGATGAT G T G C C AT A AT G G C C CGACGTGGACAAGAGTGACTGTGTTTATAGAATCTTGAACAAATTTTAAAGGCGTAATTAAATCTTGCAACTCAGATGTTATCATCAGTCTTTAGATGACAATTCGGGACTTCTATGAAGA 4320 T T C A G G A T A A A C I T TTATCGAATTGGAGGAACATTCGTTGAATTCTCACGAAGGCAACGCAGCTACTGTGGGCTAGCGGAAATAGCTCATAGTCCCGAACGTGAATTCTTAGTTCGTGGAGCCGTTGGTAGTGGTAA 4560 C G C T C T C T C T C T C T C T C T 5040 

Geonomic comparison of distinct PRSV strains

TTCGGATAATCGCATGATTAGGTACTCAAAGCAGGCAGTTAGGTTGGGAGGAGAATCCAACGACTTGGTCGGCGTTGGAAGACATAAAGAGGGAATTGCACTAAGAATCGGTCACACTGA A C C G A A C T T G A C T T G A AAAGGGCATCCAAGAAATGCGCAAGCTACTGAAGCAGCTTTCCTGAGTTTCACGTATGGCTTGCCTGTGATGACTCATAATGTGGGATTAAGTTTACTCAAAAACTGCACTGT 5520 G A T T G G T C C T C C A G C G AAGGCAAGCACGCACAATGCAACAGTACGAATTGAGTCCGTTCTTCACACAAAATTTGGTCAATTTTTGATGGCACAGTACACCCCAAGATCGGTGTGCTATTACGTCCTTACAAGTTGAG G A T C A C T A G C G T CT G C T C GATTGTGAGATCAGATTAAGTGAAGCAGCGATACCGCATGGAGGGCGATCTATTGGATGTCTGCTAGGGAGTATGAAGCAGTTGGTGGTCGTCTTTGCCTGGAAAGTGATGTTGGAAT A AG T C T A C G C G C 6000  $\begin{array}{cccc} \texttt{Accrcacgrcacgaaaacaatgttgaaaaccatgaatgcatgttgatgaagccgtttgatgaggtgttgatgaggttgatggaggttgagggagttggggtttgaaagc 6240 \\ \texttt{T} & \texttt{A} & \texttt{C} &$ CCAAGGTTTCTCCGCCGACAAGAGCAAAAAATTAAGGTTCAAATCAGCAGCAAAATGCAAAGCTTGGCCGAGAAGTTTATGGAGAGAACAATCGAGCAATATTTCGGGAGAGGCGTA 6480 T A G A T G G T T G C G T C T A 6600 AACAGGGGAGAGGCTGGATGAGAATCCTCAGACTGATATCTCAATGGTGCAGGAACACTTTGGTGACATACGAACAGTACATGGAATCGGACAGTTTCGATGGACAGTCATTAATCGC 6720 T TT A GC A C A G G T A TGATAGAGAAGCTGAGTTAAGGCAAAACAGGCCCAACGAAAACAATTTTGGTCGACGACGAAAAATCTGTTCATCATGAGGGAAAAAGTCTTTGCCAAGGCATGAGGAA C G C A G G T CAA A T G CT G A C A T A CAATGGTGAACTCATTGTGAAAATCCCAACATGGAAAGTTTGTTGAAAAACACTCACAACACTCCGGGCTAGCCCCAGTTGGCAAGACTGATCTCTTATCATTAGAATGCCGAAGGACTT 7200 T T A C C T A C C C AAA T T A T C G A T TCCACCATTCCACAGCAGAGGCAAGGTTCAGGGCCATGAAGGCTGGTGATAAGGTTTGTATGATTGGCGTTGATAATCAAGAAAATCATATTGCTAGGAAGTGTCTGAGACTTCAATCAT T T T T T A G C C A T C C G C C A A C T TCCCCTAATGGGACACTACATGAAGAGTGTTCTAAGCAAGGAAGCATACGTTAAGGATTTAATGAAATATTCAAGTGACATAATTGTTGGTGAAGTTGACCACGATGTGTTGAGGAAAG 7920 A T T G C A CA T T T TGTTGCGCAAGTTGCTGATGCTGAAACGACCACGAGGTGTCCTGAGGTTGAATACATCACAGGATGCTGCAGGTCATACAAGCTCTAAAATATGGATGCTGCTGTTGGGGCTCTATACAC 8040 A C T T T C A A C T A G T CT G C A C A CT T AGGAAAGAAAAGAAAATATTTCGAAGGATCAACGGTTGAGCACAAGGCTCTAGTGCGCAAAAGTTGTGGAGCGCTCTGTATGGAGGGCAGATGGGTGTGTGGAACGGATCATTAAAGGC 8160 G G T G A G T A T A C C C A AGA C C T GC G CGAGCTGCGACCTGCAGAGAAAGTTCTAGCCAAAAAGACAAGATCTTTCACTGCGGGGGCCCCTCGGACACTTTGTTGGGAGCCAAAGTTTGTTGGATGATTAATAATTGGTTCTACAG 8280 A A A T G C G G A T A A C T AC A A C C C C T 8400 TATTITGACACCTGATGGGACGATTGTCAAGAAATTCAAAGGAAATAATAGTGGTCAACCGCTTGAACAGTTGTTGATAACACACTGATGGTTTTAATTACAATGTATTACGCGCTACGCAA A AC G A A A C C C G T T C A G 8640 AAGAATTGTAGCCATCCTTGAGTGGGGACAAATCTAAGCTTCCAGAACATCGATGGAATGCATGGCGAATGATAGAATCATGGGGGATACGAGGGGCTAACGCACGAGATCCGCAG GC C A T A GT T A T T GATCTTACCAAGAATTAAGGGAAAAACTGTCCTTAATTTAAATCATCTTCTTCAGTATAATCCGAAACAAGTTGACATCTCCAAACACTCGCGCCACTCAATCTCAATTTGAGAAGTGGTA 9600 G TC G G G G G T T T A 9720 GATEGATEGEGEAAACCCAAGTCGATTATACCCATTAAACCTTTGATTGAACACGCAAATCCTTCATTTAGGCAAATCATEGCCACTTCAGTAACGCCGACAGGCATACATCGCGAAAGAG T A C G G T T G T T A T 9840 TGAAGCTCATATGCAGATGAAGGCTGCAGGCGCTACGCAATACTAATCGCAAAATGTTTGGAATGGACGGCAGTGCAGTAACAAGGAAGAAAACACGGAAGAACACAGTGGAAGATGT 10080 C C G A C C G G T T 

TGAGAG 10326

**Fig. 1.** Comparison of nucleotide sequences of PRSV YK (a Taiwan isolate) and HA (a Hawaii isolate). The complete sequence of the YK isolate is presented. Nucleotides of HA that differ from YK are shown below the YK sequence. Arrows indicate the initiation site of the open reading frame, the cleavage sites of viral proteins and the beginning of the 3' non-translated region of PRSV RNA. The nucleotide sequence of HA strain was from Yeh et al. [58]

MSSLYQLQPŤALKDRLLSHERGKGWIEHKLERKGERGNTRYVGEFVISEGAKILQLVQIGNTEVGRAFLEGNRRIRADIŤEIVKKTMVGRLGYDFESELWCCHSCDNTSÄKYFKKCDCGE 120 L T RAN QY R ESKK S V H CS D K T FV N IR L PI protein KYYSEGNLIKSMRDLMVQFDMTTSEIEQVGYDYLAEAVDFAEQSGVKFKAPAPEEPEIMETSASSEGGLLVASEPEIVPITTKAEEAWTIQIGEISVPLVVIKETPVISGMSRILSSTG 240 T R MRT N P NS DLE N Y LVKRSQ V E V LAM PIVA GE I MV VM V V P FSLEAEIAKPATSTVPQSEIEEAAYLALEVGNEIAEKKPELKLTPYWSASLELHKRVRKHKEHAKIEAIRVRKEKERDQRIFAALEAKLNLGTRRKGQIVVCDKRGTLKWEKCQQRKKSK 360 D T LVEKE IL E VK VH I I A Q Q R K KV S LR KS RN A TQRGH VVAQVSDSVVTKIHGNFECETRDLDVAIPGIKCATSKKMMKKOKSSKLRGSNKINYVMKNLCEIIVDRNVPVELITKRCRRSIFRKDGKNYVQLRHMSGGNAPRDVSSSPEMEKLFTRFC 480 LMQ A F Q CD G K QYSEPH QS IC PR H RIV NS I DT IE GI V K R LQ E RS N IR RQ D L Q KFLIRROSVKAARLTHGSSGLIFRPKFADRIGRYFGEYFITRGRYAGKLFDGRSKLARSVRMNMEQYNDIAEKFWLGFNRAFLRHRKPTDHVCTSDMDVTMCGEVAALATIILFPCHKIT 600 VGHKPL SKN F K NV D VV LG YAK D V LG T L' HC-Pro protein CNTCMNKVKGRVIDEVGEDLNCELERLRETLSSYGGSFGHVSTLLDQLNRVLNARNMNDGAFKEVAKKIDGKKESPWTHLTAINNTLIKGSLATGNEFGKASDSLREIVRWHLKRTESIK 720 AGSVESFRNKRSGKAHFNPALTCDNQLDRNGNFLWGERQYHAKRFFANYFEKIDHSKGYEYYSQRQNPNGIRKVAIGNLIFSTNLERFRQQMVEHHIDQGPITRECIALRNNNYVHVCSC K I V VTLDDGTPATSELKTPTKNHIVLGNSGDPKYVDLPTLESDSMYIAKKGYCYMNIFLAMLINIPENEAKDFTKRVRDLVGSKLGEWPTMLDVATCANQLIIFHPDAANAELPRILVDHRQK VV Q 960 TMHVIDSFGSVDSGYHILKANTVNQLIQFAREPLDSEMKHYIVGGEFDPTTSCLHQLIRVIYKPHELRNLLRNEPYLIVIALMSPSVLLTLFNSGAIEHALNYWIKRDQDVVEVIVLVEQ 1080 D I N S V 46K protein LCRKVTLARTILEQFNEIRQNARDIHELMDRNNKPWISYDRSLELLSVYANSQLTDEGLLKQGFSTLDPRLREAVEKTYAALLQEEWRALSLFQKLHLRYFAFKSQPSFSEYLKPKGRAD 1200 LKIVYDFSPKYCVHEVGKTLLQPIKAGAKITSHLINGCGTFIRKSVARGCAYIFKDLFQFVHVVLVLSILLQIFRSAQGIATEHIQLKQAKAEVEKQRDFDRLEALYAELCVKSGEQPTF 1320 AF L V A RI A A K L R K AFLV A RI Α ΑK EEFLDFVMEREPRLKDQVYSLIHIPVIHQAKSDNEKKLEQVIAFITLILMMIDVDKSDCVYRILNKFKGVIKSCNSDVYHQSLDDIRDFYEDKQLTIDFDITGENQINRGPVDVTFEKWV 1440 N S TN AN > CI protein DNQLSNNNTIGHYRIGGTFVEFSRSNAATVASEIAHSPEREFLVRGAVGSGKSTNLPFLLSKHGNVLLIEPTRPLCENVCKQLRSEPFHCNPTIRMRGLTAFDSTNITIMTSGFALHYYA 1560 V G G G HNIQQLRLFDFIIFDECHVIDSQAMAFYCLMEGNAIEKKILKVSATPPGREVEFSTQFPTKIVTEQSISFKQLVDNFGTGANSDVTAFADNVLVYVASYNEVDQLSKLLSDKGYLVTKID 1680 GRTMKVGKTEISTSGTK<u>S</u>KKHFIVATNIIENGVTLDIEAVIDFGMKVVPEMDSDNRMIRYSKQA<u>V</u>SFGERIQRLGRVGRHKEGIALRIGHTEKGIQEIPEMAATEAAFLSFTYGLPVMTH 1800 NVGLSLLKNCTVRQARTMQQYELSPFFTQNLVNFDGTVHPKIDVLLRPYKLRDCEIRLSEAAIPHGVQSIWMSAREYEAVGGRLCLESDVRIPFLIKDVPERLYRELWDIVQTYKRDFTF 1920 V L D G K GRINSVSAGKIAYTLRTDVYSIPRTLITIDKLIESENMKHAHFKAMTSCTGLNSSFSLLGVINTIQSRYLVDHSVENIRKLQLAKAQIQQLEAHVQENNVENLIQSLGAIRAVYHQGVDG 2040 M V S 6K VKHIKRELGLKGVWDGSLMIKDALVCGFTMAGGAMLLYQHFRDKLTSVHVFHQGFSARQRQKLRFKSAANAKLGREVYGDDGTIEHYFGEAYTKKGNKKGKMHGMGVKTRKFVATYGFKP 2160 └> NIa protein EDYSYVRYLDPLTGETLDENPQTDISMVQEHFGDIRNKYMESDSFDRQSLIANNVIKAYYVRNSAKTALEVDLTPHNPLKVCDTKLTIAGFPDREAELRQTGPPKTILVDQVPPPTKSVH 2280 S D S R D A T A N R Q S HEGKSLCQGMRNYNGIASVVCHLKNTSGKGRSLFGVGYNSFIITNRHLFKENNGELIVKSQHGKFVVKNTTTLRLAPVGKTDLLIIRMPKDFPPFHSRARFRAMKAGDKVCMIGVDYQEN 2400 K I QI HIASKVSETSIISEGMGEFGSHWISTNDGDCGNPLVSVSDGYIVGLHSLSTSTGDQNFFAKIPAFFEEKVLRRIDDLTWSKHWSYNINELSWGALKVWESRPEAIFNAQKEINQLNVFEQ 2520 T D C F Q K V SGSRWLFDKLHGNLKGVSSASSNLVTKHVVKGICPLFRSYLECNEEAKTFFNPLMGHYMKSVLSKEAYVKDLMKYSSDIIVGEVDHDVFEESVAQVVELLNDHECPELEYITDSEVIIQA 2640 └S NIb protein LINIDAAVGALYTGKKRKYFEGSTVEHRQALVRKSCERLYGGQMGVWINGSLKAELRPAEKVLAKKTRSFTAAPLDTLLGAKVCVDDFNNWFYSKNMECPWTVGMTKFYKGWDEFLRKFPDG 2760 E R K WVYCDADGSQFDSSLTPYLLNAVLSIRLWAMEDWDIGSQMLKNLYGEITYTPILTPDGTIVKKFKGNNSGQPSTVVDNTLMVLITMYYALRKAGYDTKAQEDMCVFYINGDDLCIAIHPD 2880 E HEHVLDSFSSSFAELGLKYDFNQRHRNKQDLWFMSHRGILIDDIYIPKLEPERIVAILEWDKSKLPEHRLEAITAAMIESWGYEELTHQIRRFYQWVLEQAPFNELAKQGRAPYVSEVGL 3000 A N GD  $\label{eq:rrlytskrgsmdeleavidkyferergdspellvyhesrstdnhqltrgsnthvfhqskneavdtglneklkekekqkekekqkekekdkoqdkdndgasdgndvststktgerdrdvnagtsgtf 3120 E G DY VCSN A E KE EK V Coat protein A Coa$ TVPRIKSFTDKMILPRIKGKTVLNLNHLLQYNPKQVDISNTRATQSQFEKWYEGVRNDYGLNDNEMQVMLNGLMVWCIENGTSPDISGVWVMMDGETQVDYPIKPLIEHATPSFRQIMAH 3240 V Q I H FSNAAEAYIAKRNATEKYMPRYGIKRNLTDISLARYAFDFYEVNRKTPDRAREAHMOMKAAALRNTNRKMFGMDGSVSNKEENTERHTVEDVNRDMHSLLGMRN 3344 R S R

**Fig. 2.** Comparison of the deduced amino acid sequences of PRSV YK (a Taiwan isolate) and HA (a Hawaii isolate). The complete sequence of YK is presented. Amino acids of HA that differ from YK are shown below the HA sequence. The arrows indicate the N terminus of individual putative protein processed from the polyprotein. The amino acid sequence of HA was from Yeh et al. [58]

potyviruses which contain only 6.2 to 13.1% guanine [23]. The low content of guanine in the 5' leader is a typical feature for potyviruses and other plant viruses [14]. The leader sequence of YK RNA has high AU content (70.6%), it has been proposed that this high AU content facilitates the melting of secondary structures of RNA for efficient translation [1, 22].

The 3'NTR of YK RNA contains 209 nucleotides with a poly(A) tail. A comparison of the 2960 nucleotides of the 3' region of YK RNA with those of the Hawaii severe strain HA, the mild mutant HA 5–1 and the non-papaya-infecting strain W has been previously reported [47].

# The conserved and variable regions of the 5' leader sequence

Comparison of the 5' leader sequence of YK RNA with that of HA showed that they share a 64.7% nucleotide identity. The first 23 nucleotides of both RNAs are identical and this consensus sequence was also conserved in other potyviruses [40, 58]. This highly conserved sequence may play a common and important role for potyviruses, such as the binding of VPg, the assembly initiation site of virus particle, or the polymerase recognition signal for RNA replication.

Gallie et al. [15] reported that the 5' leader of turnip yellow mosaic tymovirus (TYMV) did not enhance its translation in tobacco protoplasts, and suggested that the TYMV 5' leader is host dependent in its ability to enhance translation. Petty et al. [29] investigated the systemic movement of barley stripe mosaic hordeivirus which is influenced by a single mutation in the 5' leader sequence of the genome. They found that the systemic pathogenicity of the virus may be determined by the efficiency of translation of a viral gene in vivo and is not determined by the primary sequence of the encoded protein. Recently, the 5' leaders of the two potyviruses, PSbMV and TEV, were found to enhance GUS activity to a similar level as the 5' leaders of TMV [4, 28]. This effect on translation seems to be equivalent to the enhancing effect mediated by the TMV omega sequence, which consists of a (CAA), motif of 25 nucleotides. This motif is responsible for the translational enhancement associated with the omega sequence, and two copies of  $(CAA)_n$  alone are sufficient to confer a high level of enhancement [13]. This kind of motif is also present in the 5' leaders of YK and HA strains of PRSV, with 7 and 10 repeats of CAA motif respectively. Especially, four repeats of the CAA triplet (CAACACAACACAACAACAAAA) were found in positions 14–31 of the PRSV leaders (Fig. 1). This association may enable the leaders of PRSV to have the same effect of translational enhancement mediated by TMV omega sequence. Comparison of the 5' leaders of YK and HA from nucleotide positions 24 to 85 showed that they share only 51.6% identity (Figs. 1 and 3). This highly variable sequence may be related to a special function of PRSV, such as efficiency of translation, host determination, or pathogenicity.

# The highly variable P1 proteins

The site for hydrolyzing the P1 protein from the polyprotein of PRSV HA was previously predicted by Yeh et al. [58] to be Phe 547/Asn 548. Verchot et al. [45]

investigated the proteolytic domains of the 35kDa P1 protein of TEV by mutagenesis analysis and concluded that His 214, Asp 223, Ser 256 and Asp 288 are required for optimal autoproteolytic activity. The P1 protein of potyvirus was shown to exhibit nonspecific RNA-binding activity, the FIVRG motif of the P1 protein of potyviruses is similar to the RNP2 motif found in the RNP-CS-type binding domain. Some proteins containing this motif interact with RNA during pre-mRNA processing [3, 41]. In a recent study, the N-terminal half of P1 was shown to be dispensable for proteolysis as well as genome amplification, cell to cell movement and systemic infection [43, 44]. The cleavage site Phe/Asn and the essential amino acids for autoproteolytic activity of the TEV P1 protein are also present in the polyprotein of the YK isolate (Fig. 2).

Comparison of the P1 protein of YK with that of HA showed that they share a 70.9% nucleotide identity and a 66.7% amino acid identity. This represents 58% of the overall changes in the polyprotein, indicating that the P1 protein is more variable than the other proteins of this virus. There are only 2.63 nucleotide changes for each amino acid change in the P1 protein, but for other proteins more than 4 nucleotide changes for each amino acid difference (Fig. 3). The small ratio for the P1 protein indicates that a greater proportion of the nucleotide changes lead to amino acid changes. The P1 protein region of YK RNA contains 1 641 nucleotides and coded for a 63 kDa protein. This 63 kDa P1 protein is similar in size to that of HA and is 18 kDa to 34 kDa larger than those of other potyviruses [58]. Although the P1 proteins of PRSV YK is distantly related to those of other potyviruses, of all the P1 protein of potyviruses compared, including PRSV YK, the functional domains for serine-type proteinase activity [58] are conserved and located at the C terminal part of this protein.

Shukla and Ward [38, 39] concluded that sequence identity of the CPs between different potyviruses ranged from 38% to 71%, whereas that between strains of each species ranged from 90% to 99%. When the CP cores of more viruses were compared, the highest level of sequence identity corresponds to strains of a potyvirus species are still in 90-99% [11]. The amino acid sequence is 93.5% identical for the P1 proteins of G2 and G7 strains of SMV [21], and an even greater identity of 96.8% was found between RAN and NAT strains of PPV [24, 25]. In this investigation, only 66.7% identity was found in the P1 proteins of YK and HA. This would indicate that the two isolates are two distinct potyvirus species if the threshold for the CP identity is applied to the P1 protein. However, the CP is much more conserved among potyviruses (identity of 53.2%–56.6%) than the P1 protein (identity of 10.4%–18.3%) [58]. Therefore, the threshold of sequence similarity of CPs for grouping strains as the same potyvirus is obviously not applicable to the P1 protein. Recently, similar results were obtained when the P1 protein was compared between strains of ZYMV and PVY [42, 50]. The P1 coding region of a ZYMV strain from Reunion Island had a low (53.3–57%) amino acid sequence identity to four ZYMV strains from Florida and California [50]. Tordo et al. [42] reported that the amino acid sequence identity between 12 PVY isolates ranged from 72.8–100% in the P1 coding region, and these isolates can be classified into three geographical groups.



(b) identity are shown and the numbers in parenthesis indicate the total residues of nucleotide or amino acid different in the compared region. (a) and amino acid (b) sequences by the GAPSHOW program (GCG package, Version 7-UNIX) that showed the distribution of similarities and differences [9, 10]. The different nucleotides or amino acids are represented with vertical lines. The percentage of nucleotide (a) or amino acid Average numbers of nucleotide differences that result in one amino acid difference for each gene are indicated in (c). The nucleotide and amino acid sequences of HA strain are from Yeh et al. [58] A new standard to distinguish different strains or viruses in the potyvirus group may have to be established when more sequence information for different P1 proteins becomes available. Nevertheless, the diversity of the P1 protein should be taken into consideration in work regarding taxonomy of potyviruses.

### Other viral proteins

In previous studies of in vitro translation [8, 35, 51, 54] and the nucleotide sequence of PRSV HA, a tentative map of the polyprotein was proposed [58]. The cleavage sites in the polyprotein of YK were found to be similar to that of HA and other sequenced potyviruses (Fig. 2). Comparison of viral proteins of YK, excluding the P1 protein, with those of HA showed that they share 82.5–89.8% nucleotide identity and 91.2–97.6% amino acid identity (Fig. 3). There were 1 187 different nucleotides resulting in 133 different amino acids and a total of 42% different amino acids was found in the regions of the polyprotein excluding the P1 region. When the ratios of the number of nucleotide differences to the encoded amino acid changes were analyzed, it was found that an average of 8.92 nucleotide differences resulted in one amino acid change in regions of the polyprotein except for the P1 protein.

Comparison of the functional motifs of proteins of HA, except the P1 protein, with those of YK indicated that the motifs of the HC-Pro cysteine cluster of Cys-X<sub>8</sub>-Cys-X<sub>13</sub>-Cys-X<sub>4</sub>-Cys-X<sub>2</sub>-Cys (573–604) and the proteinase active sites of Gly- Tyr- Cys-Tyr (888–891) and His 963, and the CIP nucleotide-binding sites of GAVGSGKST (1 486–1 494) are identical. Motifs of other proteins were also conserved, as found in the VPg domain of the NIa protein (2 094 to 2 282), the protease domain of the NIa protein (2 283 to 2 520), the proteinase active sites of the NIa protein (His 2 327, Asp 2 362 and Cys 2 431) and the polymerase active sites of the NIb protein [YCDADGS(2763–2769), GNNSGQQPSTVVDNT(S)-LMV (2 826–2 842) and NGDDL-X<sub>34</sub>-K (2 869–2 908) (Fig. 2). The high degrees of conservation in the regions of the polyprotein, other than P1, and in its functional motifs indicated that YK and HA are strains of the same potyvirus.

### Divergence and conservation of the genomic RNAs of YK and HA

Most of the nucleotide differences between YK and HA occurred in degenerate codon positions allowing the amino acid residues to be conserved. For example, more than 9 nucleotide changes resulted in only one amino acid change in HC-pro, CIP, NIa and NIb proteins. The extreme one is the CIP protein in which there were 246 nucleotide differences yet only 15 amino acid changes occurred (Fig. 3). It is likely that selection for essential functions of these proteins, such as aphid transmission, RNA replication and proteolytic processing is the cause.

Besides the P1 protein, the P3, the P5 proteins and CP are also much more variable than the HC-Pro, CIP, NIa and NIb proteins; only 4.0–5.96 nucleotide changes in the former three proteins for each amino acid change (Fig. 3). There are 155 and 20 nucleotide differences in P3 and P5 that resulted in 26 and

5 amino acid changes respectively. The changed amino acids are distributed rather randomly in different areas of the P3 and P5 regions (Fig. 3). The functions of the P3 and the P5 proteins of potyviruses are still not known. Alignment of the CPs of five sequenced PRSV strains was reported by our laboratory previously [47]. Differences in CP sequence among PRSV strains were located mostly at N-terminal regions of the CPs and the variation followed the differences in geographic origins rather than host specificity. Similar results were also noticed by Bateson et al. [2] who showed that variability of the CP genes among the W and P type isolates from Australia and those from other areas is not related to host specificity, such as papaya or non-papaya infecting, but corresponding to geographic distribution. Comparison of the predicted polyproteins of YK and HA of PRSV, determined by their nucleotide and amino acid sequences, demonstrated that the P1 protein is the most variable gene product, followed by P5, CP, and P3 in order. Whether the differences in the P1 proteins are related to host specificity or corresponding to geographic distribution remains to be further investigated.

The mild strain HA 5–1 provided a high degree of protection in papaya against infection with the severe strain of PRSV in Hawaii [27]. However, different degrees of cross-protection effectiveness were observed when HA 5–1 was used against strains of PRSV in Taiwan [49]. When the mild strain HA 5–1 was used to protect papaya against the mosaic or wilt strains from Taiwan, high frequency of breakdown was observed [49, 57]. Recently, transgenic plants that expressed the CP gene of HA 5–1 [12] were tested against 13 isolates of PRSV. The results showed that the effect of strain-specific protection was similar to classical cross protection [55]. The high variations in the 5' leader sequence, in the P1 protein and in the N-terminal sequence of the CP [47] represent the major different regions between YK and HA genomes. Whether these diverged regions play a role for the differences in pathogenicity or the strain-specific protection remains to be investigated.

Although YK and HA are different strains of PRSV from distinct geographic origins, selection seems to have kept most of the viral proteins highly conserved. On the other hand, the high variability in the 5' leader region, the P1 protein and the N-terminal region of the CP suggests that the two strains may arise from different evolutionary pathways in different geographic regions. The host i.e. different papaya varieties may play an important role in providing different selection pressures for the divergence in the pathogen.

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