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Interaction between potyvirus P3 and ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) of host plants

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Abstract The P3 protein encoded by *Shallot yellow stripe virus* onion isolate (SYSV-O) interacted in the Yeast Two-hybrid (Y2H) system and in co-immunoprecipitation (Co-IP) assays with the large subunit of the ribulose-1, 5-bisphosphate carboxylase/oxygenase (RubisCO) protein that is encoded by the *rbcL* gene of its onion host. Dissection analysis by Y2H showed that the main part of SYSV P3 (amino acids 1–390) and onion RbcL (amino acids 1–137) were responsible for the interaction. The P3 proteins encoded by *Onion yellow dwarf virus* (OYDV), *Soybean mosaic virus Pinellia* isolate (SMV-P), and *Turnip mosaic virus* (TuMV) also interacted with RbcL, suggesting that a P3/RbcL interaction might exist generally for potyviruses. An interaction between P3 of these potyviruses and the small subunit of RubisCO (RbcS) was also demonstrated. Moreover, the P3N-PIPO protein encoded by a newly identified open reading frame embedded within the P3 cistron also interacted with both RbcL and RbcS. It is possible that the potyvirus P3 protein affects the normal functions of RubisCO which thus contributes to symptom development.

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The genus *Potyvirus* is one of the largest genera of plant viruses and includes many destructive pathogens. Members of this genus have a single-stranded positive-sense RNA genome containing a large open reading frame which encodes a polyprotein that is subsequently processed into 11 mature multifunctional proteins [1]. These proteins are, in order from the N-terminus of the polyprotein, P1, HC-Pro, P3, P3N-PIPO, 6K1, CI, 6K2, NIa (N-terminal VPg, C-terminal Protease), NIb, and CP [2–13]. P3, containing no recognized conserved motif or sequence, is one of the most variable potyviral proteins, and is the least studied of the major proteins [1, 14]. Here, we report that P3 proteins encoded by four potyviruses, *Shallot yellow stripe virus* onion isolate (SYSV-O), *Onion yellow dwarf virus* (OYDV), *Soybean mosaic virus* Pinellia isolate (SMV-P), and *Turnip mosaic virus* (TuMV), interacted with both large and small subunit proteins of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) of onion.

In a Yeast two-hybrid (Y2H) analysis using SYSV-P3 protein as bait and an onion cDNA library as prey, 29 independent clones were recovered following growth on selective media. Sequencing showed that 18 of these 29 clones were identical to the 5′ coding region of the *RbcL* gene encoding the plant RubisCO large subunit protein (RbcL). The complete cDNA of onion *RbcL* (Accession Number: EF613555) was then cloned for further Y2H experiments that confirmed the interaction of SYSV-O P3 with the full length onion RbcL protein (Fig. 1a). Co-immunoprecipitation (Co-IP) experiments also demonstrated the interaction in vitro (Fig. 2).

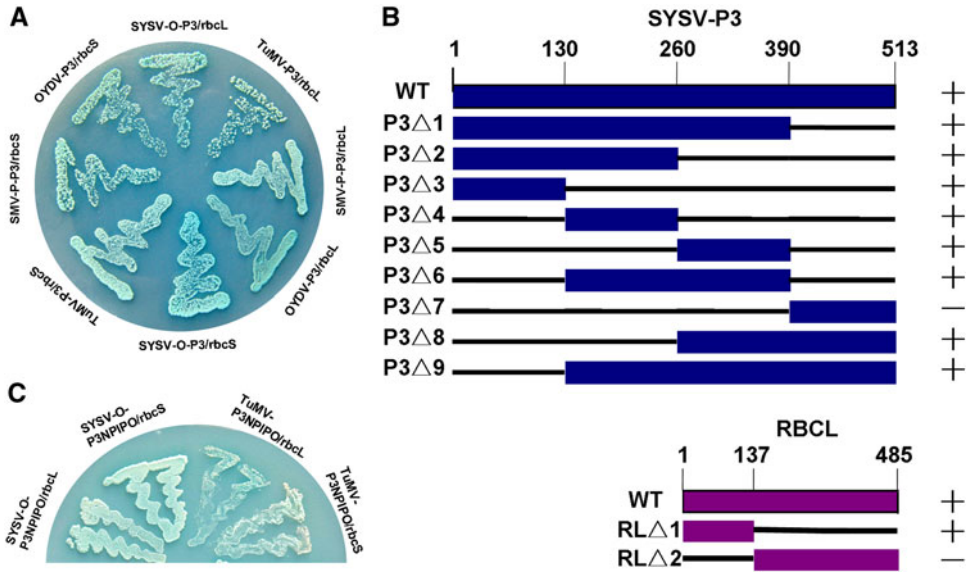

Fig. 1 Interaction of P3 proteins encoded by SYSV-O, OVDV, SMV-P, and TuMV with rbcL and rbcS. **a** Y2H results showing positive interactions demonstrated by growth of the yeast on -Trp-Leu-Ade-His/SD/X-α-gal selective medium. **b** Schematic diagram of the dissection of SYSV-P3 and RbcL proteins. The scale above each diagram shows the amino acid position and the presence (+) or absence (−) of an interaction in Y2H analyses is summarized. **c** Interaction of P3N-PIPO proteins encoded by SYSV-O, TuMV with rbcL and rbcS. Positive interactions are demonstrated by growth of the yeast on -Trp-Leu-Ade-His/SD/X-α-gal selective medium

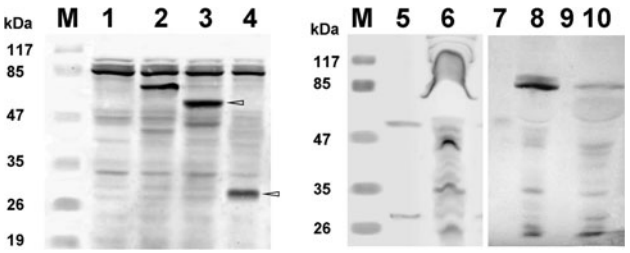

Fig. 2 Translation of SYSV-P3Δ2 and RbcL in vitro and Co-IP analysis of the interaction between the proteins. *M* Pre-stained protein molecular weight marker (Fermentas); *1* negative control for translation in Co-IP Kit, *2* positive control for translation in Co-IP Kit, *3* RbcL protein translated from pTNT-rbcL, *4* P3Δ2 protein translated from pTNT-SYSV-P3Δ2, *5* and *6* immunoprecipitate and supernatant, respectively, of the biotinylated pTNT-SYSV-P3Δ2 and pTNT-rbcL, *7* and *8* Immunoprecipitate and supernatant, respectively, of the biotinylated pTNT-rbcL without P3Δ2, *9* and *10* Immunoprecipitate and supernatant, respectively, of the biotinylated P3Δ2 without pTNT-rbcL

To identify which region of the SYSV-O P3 protein interacted with onion RbcL, the SYSV-O P3 protein was sub-divided equally into four parts (N-terminal amino acids (aa) 1–130, N-proximal 131–260 aa, C-proximal 261–390 aa and C-terminal 391–513 aa, and nine different P3 clones were produced (P3Δ1 to P3Δ9) (Fig. 1b). The onion RbcL protein was divided into two parts (N-terminal region [1–137 aa] and C-terminal region [138–486 aa]) (Fig. 1b). Y2H results showed that onion RbcL protein could interact with the major part of SYSV-O-P3 (amino acids 1–390) but not with the extreme C-terminus, and SYSV-O-P3 could interact with the N-terminal half of onion rbcL (aa 1–137) but not with remainder, which indicated that the peptide of SYSV-O-P3 at amino acids 1–390 and the N-terminus of onion rbcL were responsible for the protein–protein interaction (Fig. 1b).

To examine whether the interaction between the P3 protein and RbcL operates among other potyviruses, we analyzed the interactions between the P3 proteins of OYDV, SMV-P, and TuMV with RbcL. The *RbcL* gene is highly conserved among plants; sequence alignment showed that *RbcL* had 98% nucleotide identity between onion, tobacco, *Arabidopsis*, and rice (data not shown). Consequently, the onion RbcL protein was used in the interaction analysis rather than the *RbcL* genes from the natural hosts of OYDV, SMV-P, and TuMV. The results showed that all of the P3 proteins interacted with RbcL (Fig. 1a), suggesting that a P3/RbcL interaction might exist generally in potyviruses.

RubisCO is constructed of eight large subunits (RbcL) and eight small subunits (RbcS). The *RbcS* gene is also highly conserved in plants, with an 80% nucleotide identity existing between the onion, tobacco, *Arabidopsis*, and rice genes (data not shown). To examine whether potyvirus P3 proteins interact with the small subunit protein of RubisCO, the onion *RbcS* gene was cloned using primers designed from the published sequence (Accession number: AA451551). Y2H assays showed that the P3 proteins of SYSV-O, OYDV, SMVP, and TuMV could all interact with onion RbcS (Fig. 1a).

P3N-PIPO is a newly discovered protein of potyviruses [15]. It is expressed as an approximately 25 kDa protein containing the N-terminal part of the P3 protein and a distinct C-terminus as a result of translational frameshift. Since the N-terminal region of P3N-PIPO is the same as that of P3, it seemed probable that P3N-PIPO would also interact with RbcL. Constructs expressing the SYSV and TuMV P3N-PIPO proteins were therefore examined in Y2H assays and the results showed that both of them interacted with onion RbcL and RbcS (Fig. 1c).

Here, we have demonstrated that the P3 protein of SYSV-O interacts with the onion RbcL protein, and that there are similar interactions between RbcL and the P3 proteins of OYDV, SMV-P, and TuMV. Moreover, the P3 proteins from these viruses also interacted with the onion RbcS protein. RubisCO has also been shown to interact with the coat protein (CP) of PVY and at least four other chloroplast proteins have been reported to interact with different potyvirus proteins [16, 17]. These plant proteins, the photosystem I PSI-K protein, NtMinD (the chloroplast division-related factor), FeS (one of the cytochrome b6/f complex proteins), and FdV (the chloroplast precursor of ferredoxin-5), interacted, respectively, with the PVY CI protein, the PVY HC-Pro protein, the SMV-P P1 protein, and the SCMV HC-Pro protein [16–20]. Any or all of these interactions may help explain how infection by potyviruses decreases chlorophyll content and the photosynthetic rate of the host plant.

P3N-PIPO is necessary for viral infection. Recent work revealed that P3N-PIPO is located at the plasmodesmata and may be involved in the intercellular movement of potyviruses in infected plants [12, 21]. The interactions between P3N-PIPO and RbcS and RbcL that we have reported here suggest that it may have other functions in addition to its role in virus movement.

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