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1 Turnip mosaic virus is a second example of a virus using transmission
2 activation for plant-to-plant propagation by aphids

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14 Abstract

15 Cauliflower mosaic virus (CaMV, family *Caulimoviridae*) responds to the presence of aphid vectors
16 on infected plants by forming specific transmission morphs. This phenomenon, coined
17 transmission activation (TA), controls plant-to-plant propagation of CaMV. A fundamental
18 question is whether other viruses rely on TA. Here, we demonstrate that transmission of the
19 unrelated Turnip mosaic virus (TuMV, family *Potyviridae*) is activated by the reactive oxygen
20 species H₂O₂ and inhibited by the calcium channel blocker LaCl₃. H₂O₂-triggered TA manifested
21 itself by the induction of intermolecular cysteine bonds between viral HC-Pro molecules and by
22 formation of viral transmission complexes, composed of TuMV particles and HC-Pro that
23 mediates vector-binding. Consistently, LaCl₃ inhibited intermolecular HC-Pro cysteine bonds and
24 HC-Pro interaction with viral particles. These results show that TuMV is a second virus using TA
25 for transmission, but using an entirely different mechanism than CaMV. We propose that TuMV
26 TA requires ROS and calcium signaling and that it is operated by a redox switch.

27 Importance

28 Transmission activation, i.e. a viral response to the presence of vectors on infected hosts that
29 regulates virus acquisition and thus transmission, is an only recently described phenomenon. It
30 implies that viruses contribute actively to their transmission, something that has been shown
31 before for many other pathogens but not for viruses. However, transmission activation has been
32 described so far for only one virus, and it was unknown whether other viruses rely also on
33 transmission activation. Here we present evidence that a second virus uses transmission
34 activation, suggesting that it is a general transmission strategy.

35 Key words

36 Plant virus; aphid vector; host plant; virus transmission; virus vector host interactions; reactive
37 oxygen species; calcium; signaling

38 Abbreviations

39 CaMV, cauliflower mosaic virus; TuMV, turnip mosaic virus; TA, transmission activation; ROS,
40 reactive oxygen species; TB, transmission body; HC, helper component; HC-Pro, helper
41 component protease; CP, capsid protein

42 Introduction

43 Transmission is an obligatory step in the life cycle of parasites but it is also an Achilles's heel,
44 because parasites must leave the comparably comfortable environment of the host they are
45 installed in, and face a potentially adverse environment during the passage to a new host. Some
46 pathogens rely on resistant dormant states like spores to persist in the "wild" until they reach a
47 new host passively, e.g. carried by the wind. Most pathogens, however, actively use vectors for
48 transmission and they can manipulate both hosts and vectors in an impressive number of ways,
49 all potentially increasing transmission (1–4). In the most sophisticated cases, pathogens "*use*
50 *exquisitely controlled mechanisms of environmental sensing and developmental regulation to ensure their*
51 *transmission*" (5). This concept, implying active contribution of the pathogen, is widely accepted
52 for eukaryotic parasites (for example plasmodium, shistosoma, wucheraria, microcoelium), which
53 developed fascinating transmission cycles to control and adapt vector-host or primary-secondary
54 host interactions for their propagation (4). We have recently discovered a remarkable
55 phenomenon for a virus. Cauliflower mosaic virus (CaMV, family *Caulimoviridae*) responds to the
56 presence of aphid vectors on infected host plants by forming transmission morphs at the exact
57 time and location of the plant-aphid contact (6). This process, coined Transmission Activation or
58 TA (7), is characterized by the formation of transmission complexes between CaMV virus
59 particles and the transmission helper component (HC), the CaMV protein P2, which mediates
60 vector-binding (1). P2 and virus particles are spatially separated in infected cells since the cell's
61 pool of P2 is retained in specific cytoplasmic inclusions called transmission bodies (TBs) while
62 most virus particles are contained in another type of viral inclusion, the virus factories (8, 9). In
63 such cells, aphid punctures trigger instant disruption of TBs and the liberated P2 relocates onto
64 microtubules. Simultaneously, the virus factories release virus particles that associate with P2 on
65 the microtubules (10) to form P2/virus particle complexes, which is the virus form that aphid
66 vectors can acquire and transmit. TA is transient; P2 reforms a new TB (6) and the virus particles
67 return to virus factories (10) after aphid departure. TA implies that CaMV passes, induced by yet
68 unknown mechanisms, from a non-transmissible to a transmissible state. It has been suggested
69 that this phenomenon exists to economize host resources and to invest energy in transmission
70 only when relevant, i.e. in the presence of vectors (7). Whether this hypothesis is true or not,
71 inhibiting TA inhibits transmission, pointing to the importance of TA for CaMV. A fundamental
72 question that arises is whether TA, which is reminiscent of the active transmission strategies
73 employed by eukaryotic parasites, is exclusive to CaMV or whether it could be a general
74 phenomenon in the virus world. Therefore, we studied transmission of the turnip mosaic virus
75 (TuMV, family *Potyviridae*), which is entirely unrelated to CaMV, but uses also an HC for aphid

76 transmission. The HC of TuMV and of other potyviruses is the viral protein helper component
77 protease (HC-Pro). It is a multifunctional protein that other its HC function bears no structural,
78 functional or other similarity with P2. Our results show that TuMV is a second virus relying on
79 TA for transmission, but using a totally different mechanism.

80 **Results and discussion**

81 Signaling molecules modify TuMV transmission by aphids

82 TA requires a signaling cascade that connects the initial recognition of the presence of aphids,
83 most likely via a yet unknown elicitor, with a cellular response that is hijacked by the virus. Since
84 TA is fast for CaMV and likely also for TuMV, which uses the same transmission mode, reactive
85 oxygen species (ROS) or calcium are good signaling candidates. We therefore tested the effect of
86 the ROS signaling compound hydrogen peroxide (H_2O_2), and of a general inhibitor of calcium
87 signaling, lanthanum(III)chloride ($LaCl_3$), on TuMV transmission, using infected protoplasts as
88 virus source (6, 11). Aphid transmission tests performed with H_2O_2 -treated protoplasts showed a
89 drastic increase of TuMV transmission (Figure 1A) whereas treatment of protoplasts with $LaCl_3$
90 caused a strong reduction of transmission (Figure 1B). This effect was not due to modified cell
91 viability (Figure 1C,D). Furthermore, transmission increase by H_2O_2 and inhibition by $LaCl_3$ was
92 clearly a biological effect requiring living cells, since no effect was observed when the
93 experiments were repeated using cell extracts, i.e. dead cells (Figure 1E,F). The same control
94 experiments indicate also that H_2O_2 and $LaCl_3$ did not modify aphid feeding behavior, which
95 might have been an alternative explanation for the observed differences in transmission rates.
96 Taken together, our data show that TuMV transmission can be artificially enhanced or inhibited.

97 The protoplast system is a useful but simplified biological system because the cells are
98 individualized and not in their natural symplasmic context in a tissue. Hence, we sought to
99 validate the protoplast results by using leaves on intact infected plants as virus source. We applied
100 H_2O_2 or $LaCl_3$ to leaves by spraying treatment (12) and used these plants for aphid transmission
101 assays. To rule out any interference, only one leaf of the same developmental stage was sprayed
102 on each plant and different plants were used for each condition. H_2O_2 treatment increased
103 significantly and $LaCl_3$ treatment decreased significantly plant-to-plant transmission rates of
104 TuMV (Figure 1G,H). This confirmed the results obtained with protoplasts and showed that
105 TuMV TA is observed similarly in intact plants. Compared to the protoplast experiments, higher
106 H_2O_2 and $LaCl_3$ concentrations were required to observe significant effects. This was probably
107 due to dilution of these substances during leaf penetration. Combined, these results suggest that

108 the TA phenomenon exists for TuMV, like for CaMV, and that calcium and ROS signaling might
109 be important for TA of TuMV.

110 *The increase in virus transmission correlates with the formation of HC-Pro/TuMV transmissible complexes*

111 Next, we wanted to know how TuMV TA manifests itself in infected cells. TA of CaMV is
112 characterized by relocalization of CaMV particles and CaMV helper protein P2 from viral
113 inclusions to microtubules (6, 10). Therefore, we performed immunofluorescence experiments on
114 TuMV-infected protoplasts with antibodies directed against HC-Pro and the viral capsid protein
115 CP to determine whether H₂O₂ and LaCl₃ induced relocalization of TuMV virus particles and/or
116 HC-Pro. In untreated cells, HC-Pro and CP localized in the cytoplasm as reported for other
117 potyviruses (13, 14). Treatment with H₂O₂ and LaCl₃ did not induce any visible rearrangement of
118 HC-Pro or of CP (Figure 2). Thus, TA of TuMV is not characterized by the redistribution of
119 HC-Pro and/or virus particles within infected cells.

120 We thus hypothesized that HC-Pro and virus particles, both evenly distributed in the cytoplasm,
121 could pass from a non-associated state to an associated state, i.e. to transmissible HC-Pro-virion
122 complexes, upon TA. To visualize such complexes *in situ*, we resorted to the Duolink® technique
123 (15), an antibody-based version of the proximity ligation assay allowing detection of
124 intermolecular interactions. Duolink® performed with HC-Pro and CP antibodies showed that
125 H₂O₂ treatment indeed increased the number and intensity of HC-Pro/CP interaction spots
126 (Figure 3A,B), indicative of binding of HC-Pro to virus particles. Interestingly, incubation of
127 protoplasts with LaCl₃ decreased the number of transmissible complexes (Figure 3C). Thus, the
128 increase and decrease of HC-Pro/CP interactions, triggered by application of ROS or of a
129 calcium channel blocker, respectively, correlated with an increase and decrease of transmission
130 (compare Figures 1 and 3).

131 *Transmission activation of TuMV is characterized by formation of cysteine bridges between HC-Pro molecules*

132 We wanted to understand how HC-Pro and virus particles could rapidly transit from “free” to
133 virus-associated forms. Since ROS like H₂O₂ change directly or indirectly the cellular redox
134 potential, the formation of HC-Pro/TuMV transmissible complexes might be controlled by the
135 redox state of HC-Pro and CP, both of which contain cysteine residues that can form disulfide
136 bridges under oxidizing conditions. Therefore, we performed non-reducing SDS-PAGE/Western
137 blots to detect HC-Pro and CP migration profiles altered by intramolecular or intermolecular
138 cysteine disulfide bridges. H₂O₂ and LaCl₃ did not modify the migration profile of CP (Figure 4A).
139 However, H₂O₂ treatment increased the amount of oligomeric HC-Pro and especially of its

140 dimeric form (Figure 4B) that was previously reported to be active in transmission (16–18). LaCl_3
141 treatment had the inverse effect and decreased the amount of HC-Pro oligomers (Figure 4B).
142 The effect of H_2O_2 was concentration-dependent and clearly visible using physiologic H_2O_2
143 concentrations (0.25 mM, Figure 4C). Thus, the increase in transmission induced by H_2O_2
144 correlated not solely with formation of HC-Pro/TuMV complexes, but also with the appearance
145 of HC-Pro oligomers hold together by intermolecular cysteine bridges.

146 To have a biological significance, HC-Pro oligomerization should be completed within the
147 duration of an aphid puncture, i.e. within seconds. Kinetics of formation and breakup of HC-Pro
148 oligomers showed that both occurred within 5 seconds of incubation with H_2O_2 and LaCl_3 ,
149 respectively (Figure 4D,E). The effect of both treatments was transient because HC-Pro
150 oligomers disappeared (H_2O_2) or reappeared (LaCl_3) after ~30 min incubation. Furthermore,
151 removal of H_2O_2 by washing protoplasts showed reversibility of HC-Pro oligomerization (Figure
152 4D). Induction of HC-Pro oligomers by H_2O_2 was not restricted to TuMV or to turnip hosts,
153 because experiments with lettuce protoplasts infected with another potyvirus, *Lettuce mosaic virus*,
154 yielded similar results (not shown).

155 To better establish that formation of disulfide bridges between HC-Pro monomers contributes to
156 oligomerization, infected protoplasts were treated with the disulfide bonds-reducing agent
157 dithiothreitol (DTT) or with N-ethylmaleimide (NEM) that does not break existing disulfide
158 bridges but prevents formation of new ones by blocking free thiols. Figure 4F shows that DTT
159 treatment abolished appearance of H_2O_2 -induced HC-Pro oligomers in SDS-PAGE/Western
160 blot. This confirmed that oligomerization of HC-Pro requires establishment of intermolecular
161 disulfide bridges. NEM treatment blocked the appearance of HC-Pro oligomers in SDS-
162 PAGE/Western blots when applied before the H_2O_2 treatment, but NEM did not prevent their
163 appearance when applied after H_2O_2 treatment (Figure 4G). This is a further confirmation of the
164 involvement of disulfide bridges in HC-Pro oligomerization. Note that NEM treatment caused a
165 mobility shift of HC-Pro. This might have been due to disulfide shuffling during denaturation of
166 the samples as reported for papilloma virus (19). To establish a direct role of intermolecular HC-
167 Pro disulfide bonds in TuMV transmission, we performed transmission assays. Because of the
168 toxicity of NEM, we did not use plants as virus source but resorted to the protoplast system
169 where exposure of aphids (and the experimenter) to the substance is minimized by confining it in
170 the protoplast medium. The NEM treatment reduced virus transmission drastically (Figure 4H)
171 but did not affect protoplast viability (Figure 4I), suggesting that *de novo* formation of

172 intermolecular HC-Pro disulfide bonds is required for formation of transmissible complexes and
173 thus for aphid acquisition of TuMV.

174 *Model of TuMV transmission activation*

175 In this study, we demonstrate that TA exists for a second virus, TuMV. TuMV TA was induced
176 by the ROS H_2O_2 and inhibited by the calcium channel blocker $LaCl_3$, ROS and calcium signaling
177 are both important in early perception of parasites including insects (20) and recently aphid
178 punctures were described to induce rapid calcium elevations around feeding sites (21). Since ROS
179 and calcium signaling are often interconnected (22, 23), TuMV TA likely hijacks an early step of
180 at least one of these pathways. The initial eliciting event remains unknown. It might be a direct
181 effect of aphid saliva-contained ROS or ROS-producing peroxidases (24) that are injected into
182 cells during feeding activity. Alternatively, an aphid or aphid-induced plant factor might interact
183 in a classic pathogen-associated molecular pattern (PAMP)-triggered immunity reaction with a
184 pattern recognition receptor (PRR) (25) that prompts calcium and ROS mediated downstream
185 events. Interestingly, a recent study has demonstrated that the red clover necrotic mosaic virus
186 (RCNMV) requires ROS for replication (26). The authors proposed that plant viruses may have
187 evolved a complex mechanism to manipulate the ROS-generating machinery of plants to
188 improve their infectivity, or, transferred to this case, transmission.

189 TA of TuMV manifests itself by creation of HC-Pro intermolecular disulfide bridges, driven by
190 oxidation of the cellular redox potential. We propose that oxidation of HC-Pro induces a
191 functional switch rendering HC-Pro able to interact with virus particles and form transmissible
192 complexes (Figure 5). Functional switching (moonlighting) by redox-driven modification of
193 disulfide bridges has been reported for other proteins and is operated by conformation changes
194 affecting the secondary, tertiary or quaternary structure of proteins (27–29). Why would there be
195 such a switch? HC-Pro is a multifunctional protein involved not only in aphid transmission (30)
196 but also in pathogenicity (31), viral movement (32) and suppression of plant RNA silencing (33–
197 35). One (or more) functional switches could assist to coordinate these multiple functions by
198 allowing interaction with virions and formation of transmissible complexes only when
199 transmission is possible, i.e. when the aphids puncture cells This would help to economize finite
200 plant resources as proposed earlier (7).

201 Unfortunately, we cannot provide an empirical proof that the aphid punctures directly trigger
202 TuMV TA. In contrast to CaMV, where TA was directly visible using qualitative
203 immunofluorescence observation of P2 and virus particle networks (the characteristic

204 manifestation of CaMV TA) in cells in contact with aphid saliva sheaths, TuMV TA cannot be
205 revealed by a qualitative analysis. The quantitative Duolink® approach we used to demonstrate
206 TuMV HC-Pro/CP interactions in protoplasts required an enormous number of cells for analysis
207 and statistical validation. Identifying a comparable number of cells in tissue and in contact with
208 aphid stylets is barely feasible. The same restrictions apply to electron microscopy techniques to
209 localize HC-Pro on virus particles by immunogold labeling. Thus, proof of aphid implication in
210 TA of TuMV remains indirect, for the time being.

211 Nonetheless, we here demonstrate TA for a second virus, TuMV, different from CaMV,
212 suggesting that transmission activation might be a more general phenomenon. The great
213 phylogenetic distance between TuMV and CaMV makes it likely that the phenomenon of TA
214 arose independently for the two viruses during evolution. An obvious question is whether yet
215 other viruses use TA for their transmission.

216 **Materials and methods**

217 Plants, viruses and inoculation

218 Turnip plants (*Brassica rapa* cv. Just Right) and lettuce (*Lactuca sativa* cv. Mantilla and Trocadero)
219 were grown in a greenhouse at 24/15 °C day/night with a 14/10 h day/night photoperiod. Two-
220 weeks-old turnip plants were mechanically inoculated with wild-type TuMV strain C42J (36), and
221 two-weeks-old lettuce plants with *Lettuce mosaic virus* (LMV) strain E (37). Plants were used for
222 experiments at 14 days post inoculation (dpi).

223 Isolation of protoplasts

224 Protoplasts from turnip leaves were obtained by enzymatic digestion as described (6).

225 Preparation of infected cell extracts

226 TuMV-infected turnip protoplasts were sedimented and resuspended in SAKO buffer (500 mM
227 KPO₄ and 10 mM MgCl₂ pH 8.5) (38). Then sucrose was added to a final concentration of 15 %
228 and the suspension was vortexed to homogenize protoplasts.

229 Drug treatments and cell viability assay

230 For drug treatments of protoplasts, the following substances were added from stock solutions for
231 the indicated times to 500 µl of protoplast suspension: 1 mM LaCl₃ (5 min), 2 mM H₂O₂ (5 min),

232 3 mM NEM (20 min), 5 mM DTT (30 min). Protoplasts were incubated at room temperature
233 with gentle stirring (5 rpm). 15 min after treatments, protoplast viability was determined with the
234 FDA test (39). For drug treatments of plants, one leaf per plant was sprayed with 10 mM LaCl₃,
235 20 mM H₂O₂ or with water, and the leaf, still attached to the plant, used for transmission
236 experiments after the applied solutions had evaporated.

237 Aphid transmission tests

238 A nonviruliferous clonal *Myzus persicae* colony was reared under controlled conditions (22/18 °C
239 day/night with a photoperiod of 14/10 h day/night) on eggplant. The transmission tests using
240 protoplasts were performed as described (6), with an acquisition access period of 15 min and
241 transferring 10 aphids to each test plant. For plant-to-plant transmission tests, an acquisition time
242 of 2 min was used and only one aphid was transferred on each turnip plant for inoculation.
243 Infected plants were identified by visual inspection for symptoms 3 weeks after inoculation.

244 Antisera

245 The following primary antibodies were used: commercial rabbit anti-TuMV (sediag.com) and
246 mouse and rabbit anti-HC-Pro (recognizing HC-Pro from different potyviruses, produced against
247 the conserved peptide SEIKMPTKHHHLVIGNSGDPKYIDL P by proteogenix.fr and
248 eurogentec.com, respectively). The following secondary antibodies were used: Alexa Fluor 488
249 and 594 anti-rabbit and anti-mouse conjugates (thermofisher.com) for immunofluorescence, anti-
250 rabbit IgG conjugated to alkaline phosphatase (www.sigmaaldrich.com) for western blotting and
251 corresponding Minus and Plus probes (www.sigmaaldrich.com) for Duolink®.

252 Immunofluorescence

253 Protoplasts were fixed with 1 % glutaraldehyde and processed as described (6). The primary and
254 secondary antibodies were used at 1:100 and 1:200 dilutions, respectively.

255 Western blotting

256 Drug treatments of protoplasts were stopped by lysing protoplasts in non-reducing 2x Laemmli
257 buffer (v/v) (40) except where indicated otherwise. Optionally, oligomer formation was stabilized
258 by incubating protoplasts with 3 mM NEM for 20 min before lysis. This step yielded sharper
259 oligomer bands. Samples were then resolved by 10 % SDS-PAGE. Proteins were transferred to
260 nitrocellulose membranes and incubated with primary and secondary antibodies as described (6)

261 except that TuMV-specific primary antibodies (1:1000 dilution) were used. Antigens were then
262 revealed by the NBT/BCIP reaction. Equal protein charge on the membranes was verified by
263 coloring the RuBisCO with Ponceau S Red.

264 Duolink® proximity ligation assay

265 *In situ* protein/protein interactions were detected by proximity ligation assay using the Duolink®
266 kit (www.sigmaaldrich.com). Protoplasts were isolated from healthy or infected (14 dpi) turnip
267 leaves and fixed with 3 % paraformaldehyde in 100 mM cacodylate buffer (pH 7.2) or 100 mM
268 phosphate buffer (pH 7.4). The fixed protoplasts were immobilized on L-polylysine-coated slides.
269 Antibody incubation with rabbit anti-TuMV and mouse anti-HC-Pro, ligation and probe
270 amplification were performed according to the manufacturer's instructions. The slides were
271 mounted with Duolink® *in situ* mounting medium with DAPI (www.sigmaaldrich.com).

272 Microscopy

273 Immunolabeled protoplasts were observed with an Olympus BX60 epifluorescence microscope
274 (olympus-lifescience.com) equipped with GFP and Texas Red narrow band filters and images
275 acquired with a color camera. Duolink® images were acquired with a Zeiss LSM700 confocal
276 microscope (zeiss.com) operated in sequential mode. DAPI was excited with the 405 nm laser and
277 fluorescence collected from 405-500 nm, Duolink® probes and chlorophyll were excited with the
278 488 nm laser and fluorescence collected from 490-540 nm (Duolink® signal) or from 560-735 nm
279 (chlorophyll autofluorescence). Raw images were processed using ZEN or ImageJ software.
280 Quantification of Duolink® interactions was performed on maximum intensity projections with
281 the Analyse_Spots_Per_Protoplast macro for ImageJ, developed for this experiment (41).

282 Statistical analysis

283 Statistics and box plots were calculated with R software version 3.4.0 (r-project.org).
284 Transmission rates and cell viability were analyzed with generalized linear models (GLM). Quasi-
285 binomial distributions were used in order to take overdispersion into account, and p-values were
286 corrected with the Holm method (42) to account for multiple comparisons.

287 Analyzing the Duolink® experiments required the calculation of the total fluorescence intensity
288 (F_{tot}) of labeled foci as:

289

290
$$F_{tot} = \frac{n \times \bar{s} \times \bar{I}}{A}$$

291

292 where n is the number of labeled foci, \bar{s} the average size of a focus, \bar{I} the average fluorescence
293 intensity of a focus and A the size of the protoplast. F_{tot} was log-transformed (to normalize the
294 distribution) and analyzed with linear models using “treatment” and “replicate” as categorical
295 explanatory variables.

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398 aphids and arboviruses. *Plant Signal Behav* 8:e24225.

399 **Acknowledgments**

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408 **Legends to Figures**

409 **Figure 1.** Effect of H₂O₂ and LaCl₃ on TuMV transmission by aphids. (A-B) Turnip protoplasts
410 were incubated for 5 min with 2 mM H₂O₂ (A) or 1 mM LaCl₃ (B) and then employed in
411 transmission assays. (C-D) Cell viability of protoplasts was measured to determine if the altered
412 transmission rates were due to modified viability. (E-F) Cell extracts from protoplasts were
413 treated identically with H₂O₂ (C) or LaCl₃ (D) and used in transmission assays. (G-H) Leaves on
414 intact plants were sprayed with 20 mM H₂O₂ (E) or 10 mM LaCl₃ (F) and then employed in
415 transmission assays. Means of infected test plants (horizontal black bars in the box plots) are
416 calculated from a pool of three independent experiments in which a total of 360 tests plants were
417 used per condition. Each experiment had 6 repetitions for each condition and 20 tests plants per
418 repetition (see Supplementary Data Set S1 for raw data). *p* designates p-values obtained by
419 generalized linear models (see materials and methods). The box plots here and in the other
420 figures present medians, upper and lower quartiles, the ends of the whiskers present lowest and
421 highest datum still within 1.5 IQR of the lower and higher quartile, respectively, and the circles
422 show outliers.

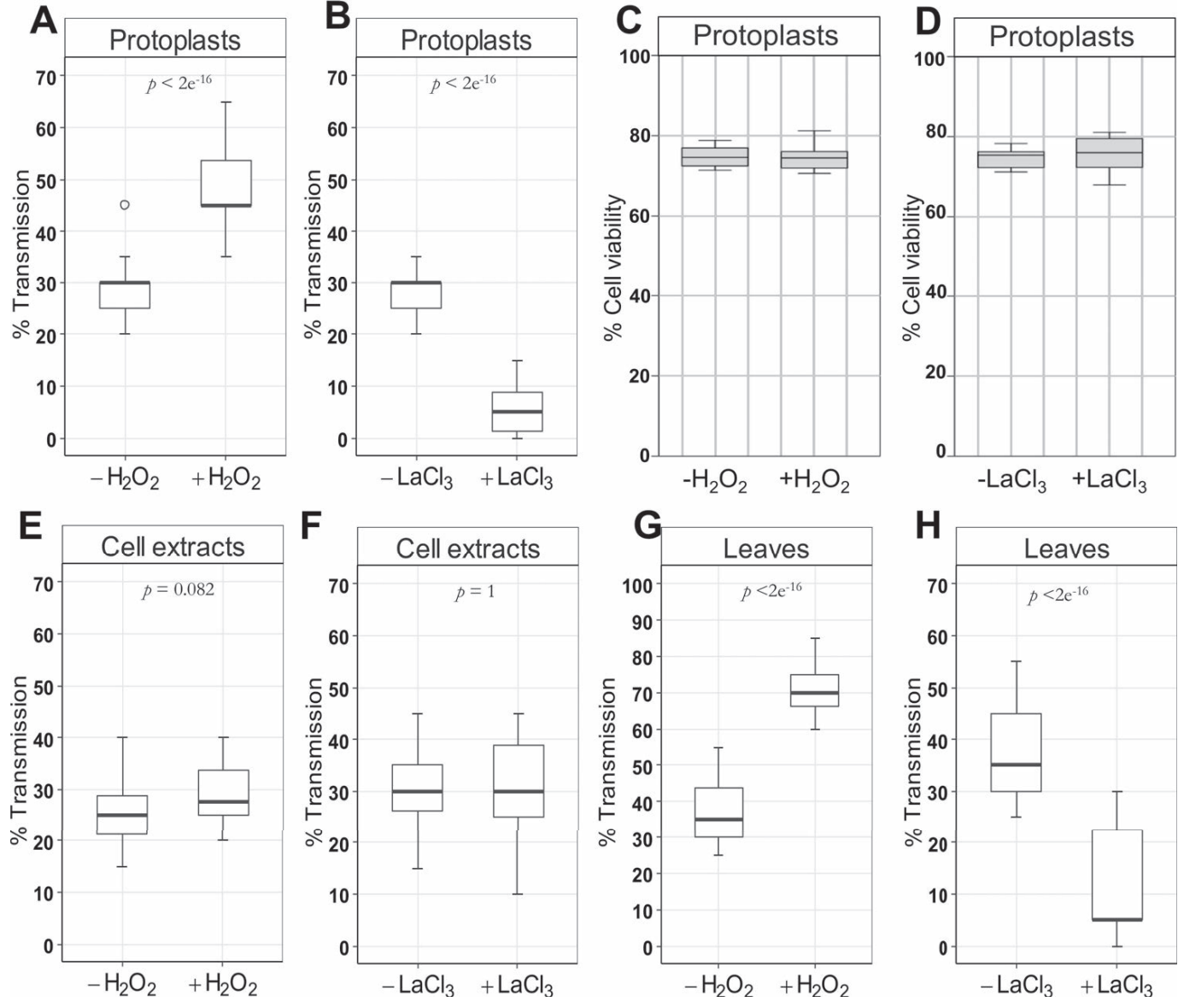
423 **Figure 2.** Immunofluorescence of turnip protoplasts infected with TuMV. TuMV-infected
424 protoplasts were treated as indicated and double-labeled against HC-Pro (green, first column) and
425 viral capsid protein CP (red, middle column). The right column (Merge) represents superposition
426 of HC-Pro and CP labels, with co-labeling appearing in yellow. Control, untreated protoplasts;
427 H₂O₂, incubation with 2 mM H₂O₂ for 15 min, LaCl₃, incubation with 1 mM LaCl₃ for 15 min.
428 Scale bars 50 μm.

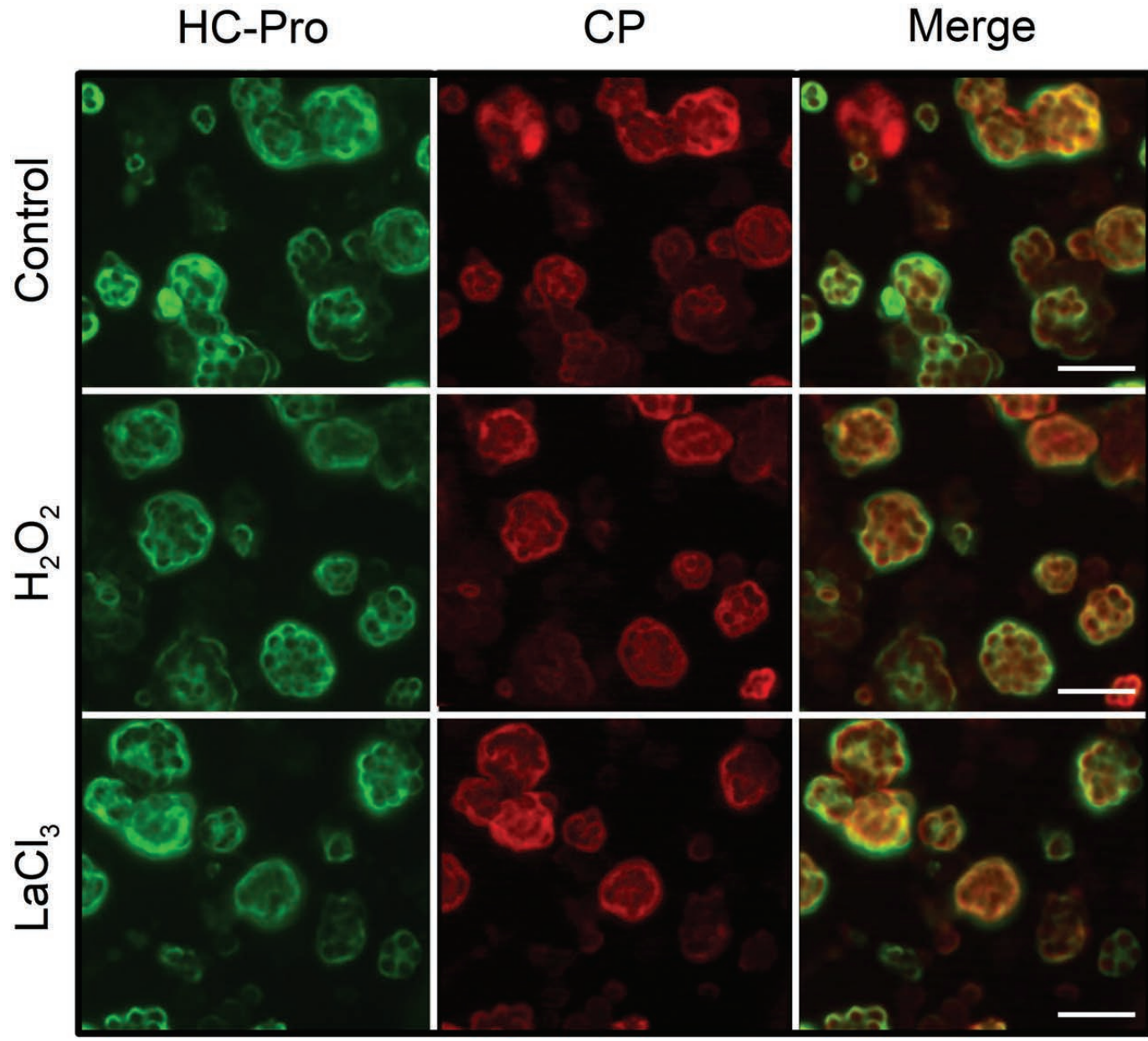
429 **Figure 3.** *In situ* Duolink® proximity ligation assay on turnip protoplasts infected with TuMV.
430 (A) Untreated control protoplasts or protoplasts incubated with either H₂O₂ or LaCl₃ were
431 processed by Duolink® for detection of HC-Pro/TuMV particle interactions using HC-Pro and
432 CP antibodies and corresponding Duolink® probes. Interactions are visible as green fluorescing
433 spots. Nuclei were counterstained with DAPI (blue) and chloroplast autofluorescence is
434 presented in grey to reveal the cell lumen. Scale bars: 20 μm. (B-C) Quantitative analysis of the
435 Duolink® signal shows that (B) H₂O₂ increased and (C) LaCl₃ decreased HC-Pro/CP interactions.
436 The box plots presents data from three independent experiments using between 56-115
437 protoplasts for each condition. The y-axes show HC-Pro/CP interactions, presented as total
438 fluorescent intensity (F_{int}). *p* designates p -values obtained by generalized linear models (see
439 material and methods).

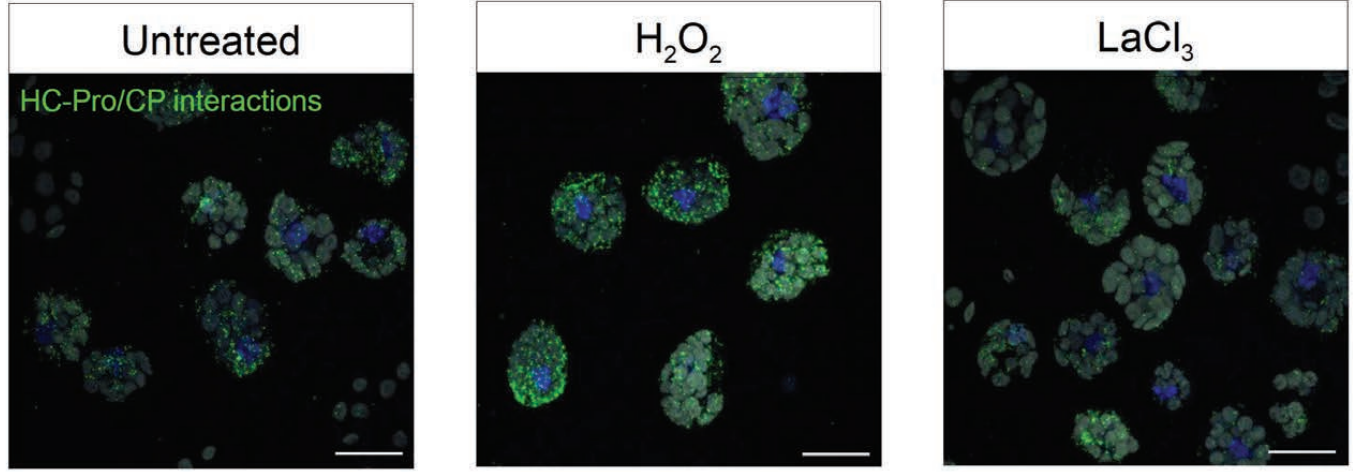
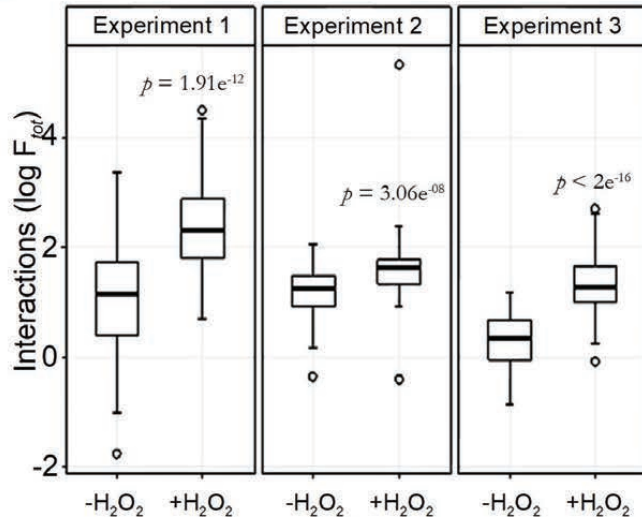
440 **Figure 4.** Non-reducing SDS-PAGE/Western blotting analysis of HC-Pro and CP from TuMV-
441 infected turnip protoplasts. The samples were lysed in a buffer without reducing agents to
442 conserve the disulfide bridges. (A) H_2O_2 and $LaCl_3$ treatments did not modify the migration
443 profile of the capsid protein (CP), whereas they (B) induced (H_2O_2) or inhibited ($LaCl_3$)
444 formation of HC-Pro oligomers. (C-D) The concentration range and the kinetics of H_2O_2
445 incubation shows that HC-Pro oligomerization (C) was induced by a minimum concentration of
446 0.25 mM and (D) that it was rapid and reversible, either by extended H_2O_2 treatment (left panel)
447 or by washing protoplasts (right panel). (E) Also inhibition of HC-Pro oligomerization by $LaCl_3$
448 was rapid and reversible. (F-G) HC-Pro oligomers are formed by intermolecular disulfide bridges
449 because (F) incubation of protoplasts with DTT, either alone or after H_2O_2 treatment, abolished
450 HC-Pro oligomers, and (G) treatment with NEM before but not after previous incubation with
451 H_2O_2 prevented their formation. (H) Transmission tests using NEM-treated protoplasts show a
452 drastic diminution of TuMV transmission. Transmission tests were performed three times using
453 320 plants per condition and analyzed by generalized linear models as described in Figure 1. (I)
454 Protoplast viability assays show that NEM treatment did not change cell viability under the
455 conditions used. TuMV, samples of TuMV-infected protoplasts; Non inf., samples of non
456 infected protoplasts; $LaCl_3$, treatment with 1 mM $LaCl_3$ for 5 min; H_2O_2 , treatment with 2 mM
457 H_2O_2 for 5 min; wash, H_2O_2 was removed by centrifugation and resuspension of protoplasts in
458 fresh medium; DTT, treatment with 5 mM DTT for 30 min; NEM, treatment with 3 mM NEM
459 for 20 min. Equal loading of lanes is shown by Ponceau Red staining of the large RuBisCO
460 subunit (Rub). A precolored ladder and the molecular masses in kDa are indicated at one side of
461 each blot. p in (H) designates p-value obtained by generalized linear models from three
462 independent experiments.

463 **Figure 5.** Model of TuMV acquisition by aphids. For simplicity, aphids, viral components and
464 the plant cell are not drawn to scale. (1) Before the arrival of aphid vectors, the redox potential of
465 the cytosol of TuMV-infected cells has ‘normal’ values, i.e. it is reduced. Consequently, the
466 cytosolic HC-Pro protein (blue circles) is in a reduced form (the red points in HC-Pro present
467 reduced cysteines) and contains no intermolecular disulfide bridges. This form of HC-Pro is
468 presumably not associated with virus particles (purple lines). It is likely but remains to be
469 confirmed whether reduced HC-Pro is dimeric as presented here. (2) When an aphid feeds on a
470 leaf infected with TuMV, an unknown elicitor is recognized by the plant cell and induces the
471 opening of calcium channels (pink cylinder) and triggers directly or indirectly ROS production in
472 the cell. During this activation stage, the ROS in the cytoplasm increases (red lightning) the redox
473 potential of the cell cytoplasm and oxidizes one or more HC-Pro cysteines. This oxidation

474 generates disulfide bridges (red lines) between different HC-Pro molecules. The intermolecular
475 disulfide bridges either induce oligomerization of a portion of HC-Pro or change the
476 conformation of a part of existing oligomers, presented by the transition of the circles to squares.
477 For simplicity, higher HC-Pro forms are not shown. (3) Whatever the case, oxidation of a
478 fraction of HC-Pro results in a functional switch of the protein and the oxidized tertiary or
479 quaternary conformation allows interaction between HC-Pro and TuMV particles and the
480 formation of TuMV transmissible complexes, symbolized by square HC-Pro aligned with a virion.
481 Now the infected cell is switched into transmission mode and this stage allows efficient
482 acquisition of TuMV. (4) The aphid acquires transmissible complexes and transmits the TuMV
483 during the next punctures on another plant. After vector departure, the redox potential of cell
484 cytoplasm lowers again and HC-Pro is reduced. This changes its conformation and induces
485 dissociation of the transmissible complexes, leaving HC-Pro free to fulfill its other functions
486 during infection. The aphid drawing is modified from (43), published under open CC3.0 license.





A**B****C**