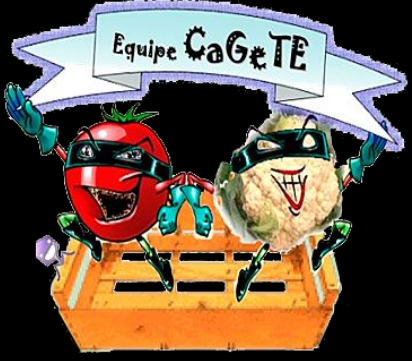


# Cauliflower mosaic virus prepares its transmission



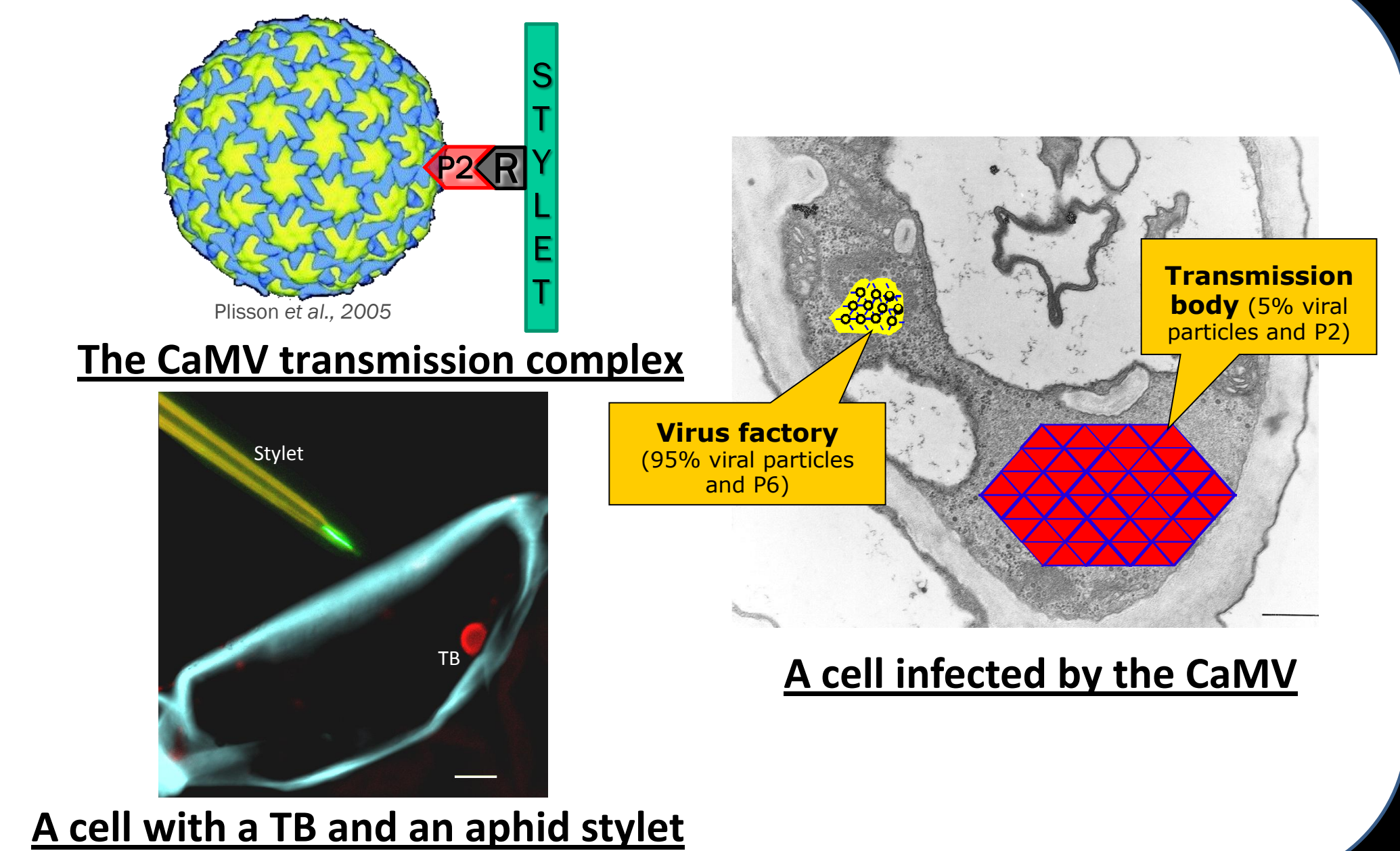
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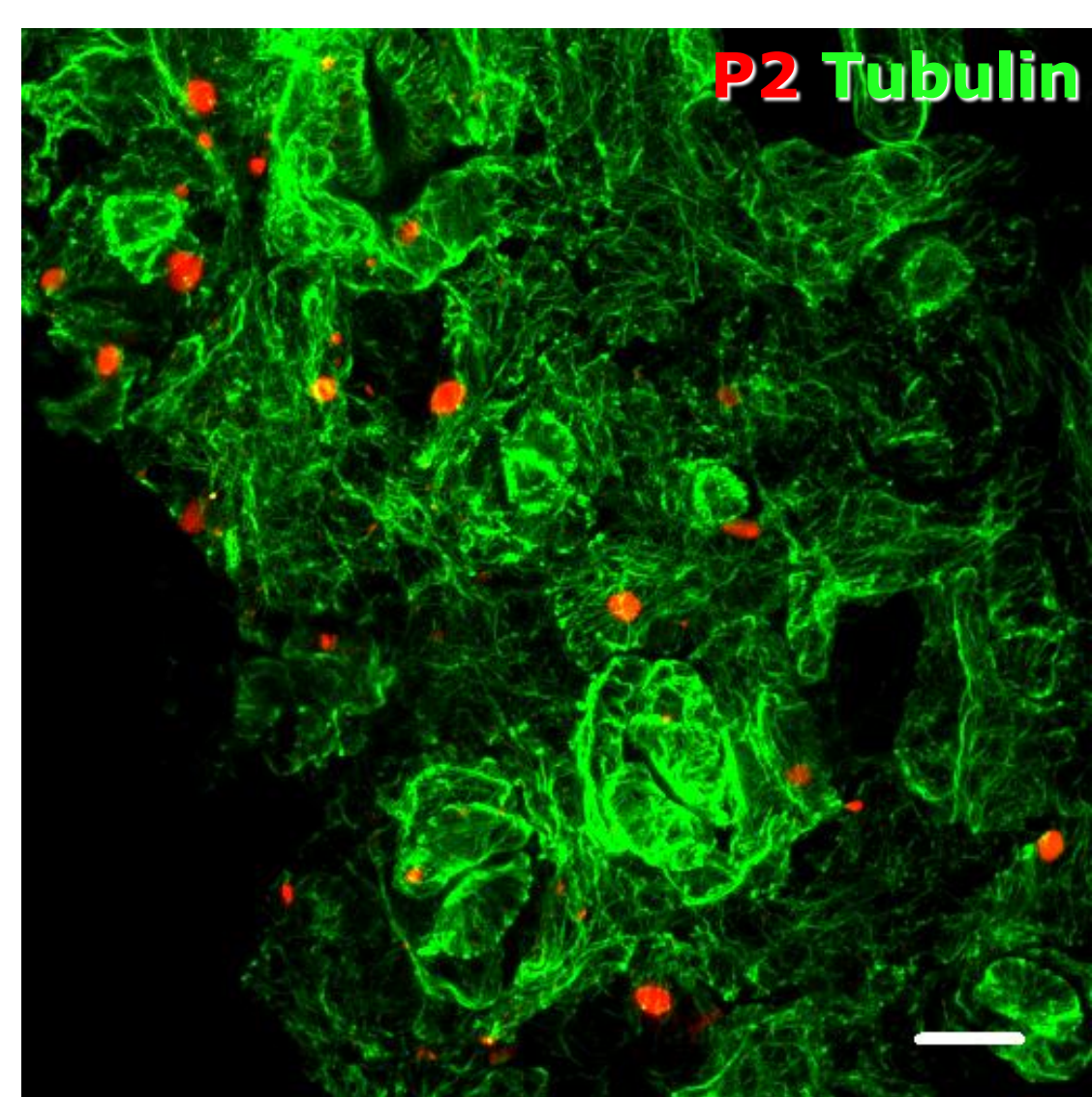
## PROBLEM

*Cauliflower mosaic virus* (CaMV) is transmitted by aphids. CaMV forms in infected cells many viral factories, which contain a lot of CaMV particles, and a single “transmission body” (TB). The TB contains only few viral particles and the aphid transmission protein P2. P2 is absolutely required for virus transmission because it binds CaMV particles to a receptor localized in the stylets of the aphid vector. However, P2 is sequestered in the TB, inaccessible for the aphid. How can P2 be efficiently acquired by the aphid? The TB “detects”, via the cell, the aphid attack by yet unknown mechanisms and passes from an inactive state to an active state where P2 from the TB and viral particles from unknown origin redistribute on microtubules, rendering P2 and viral particles accessible to the aphid. We are studying the functioning of the TB and we are trying to understand the origin of the viral particles relocating on microtubules.

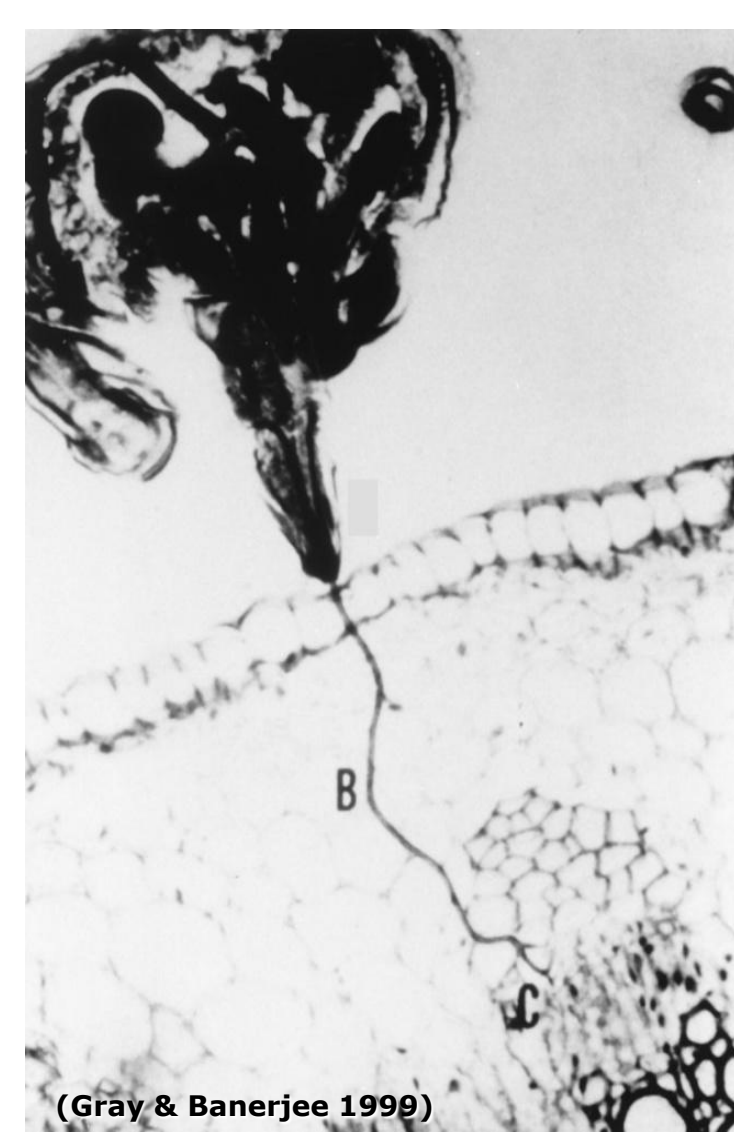


## RESULTS

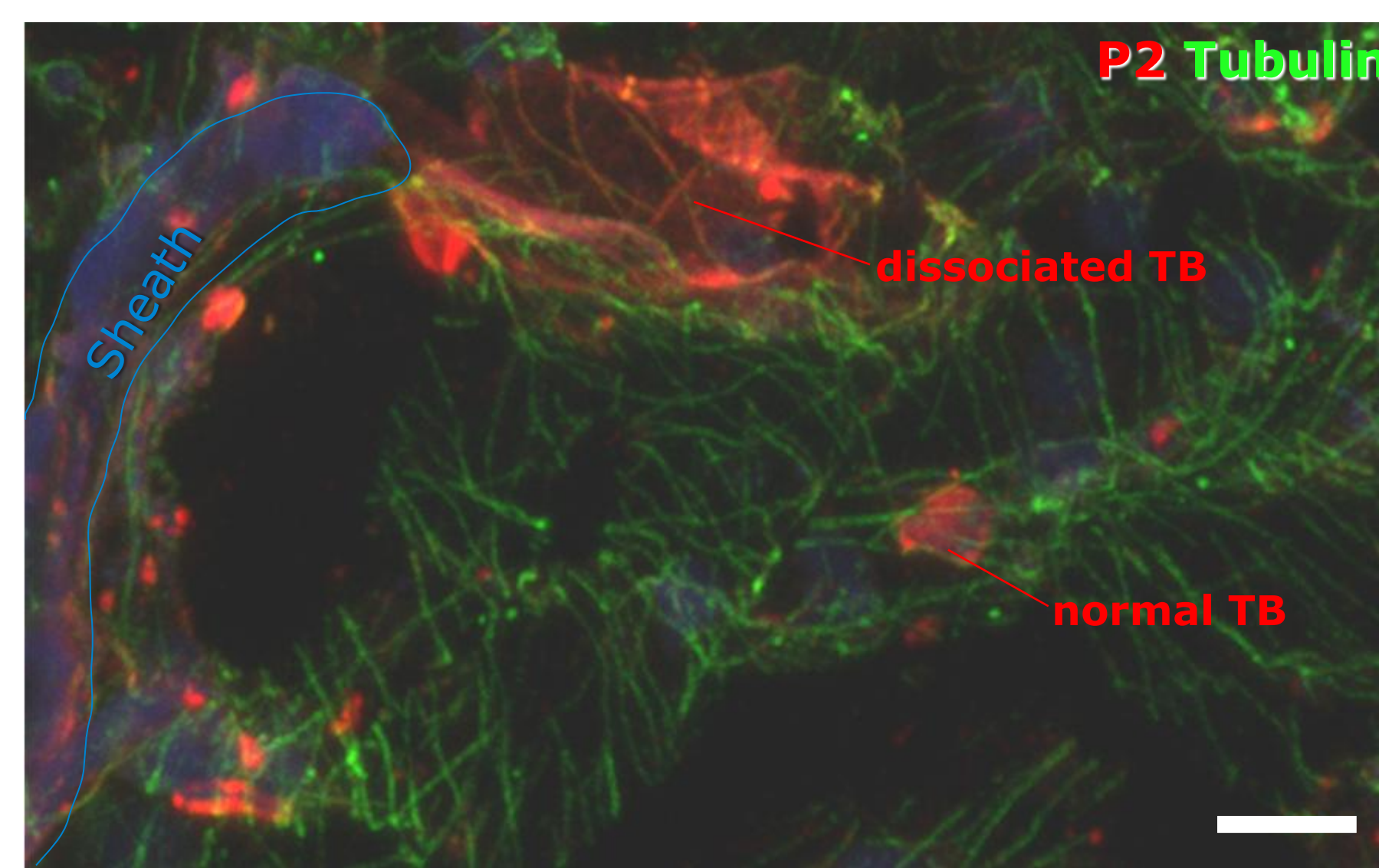
### Aphids trigger TB activation



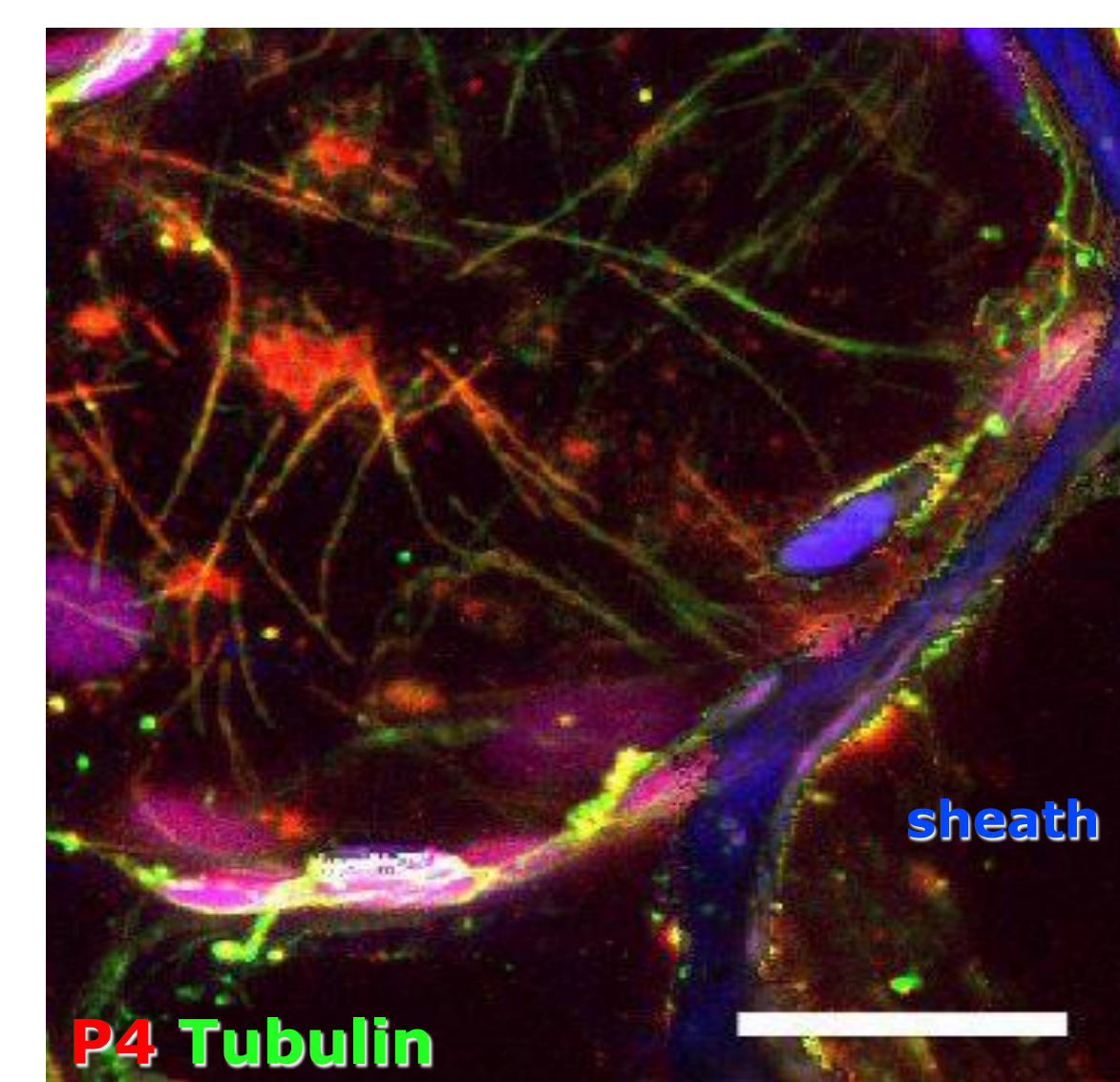
(1) Infected control tissue



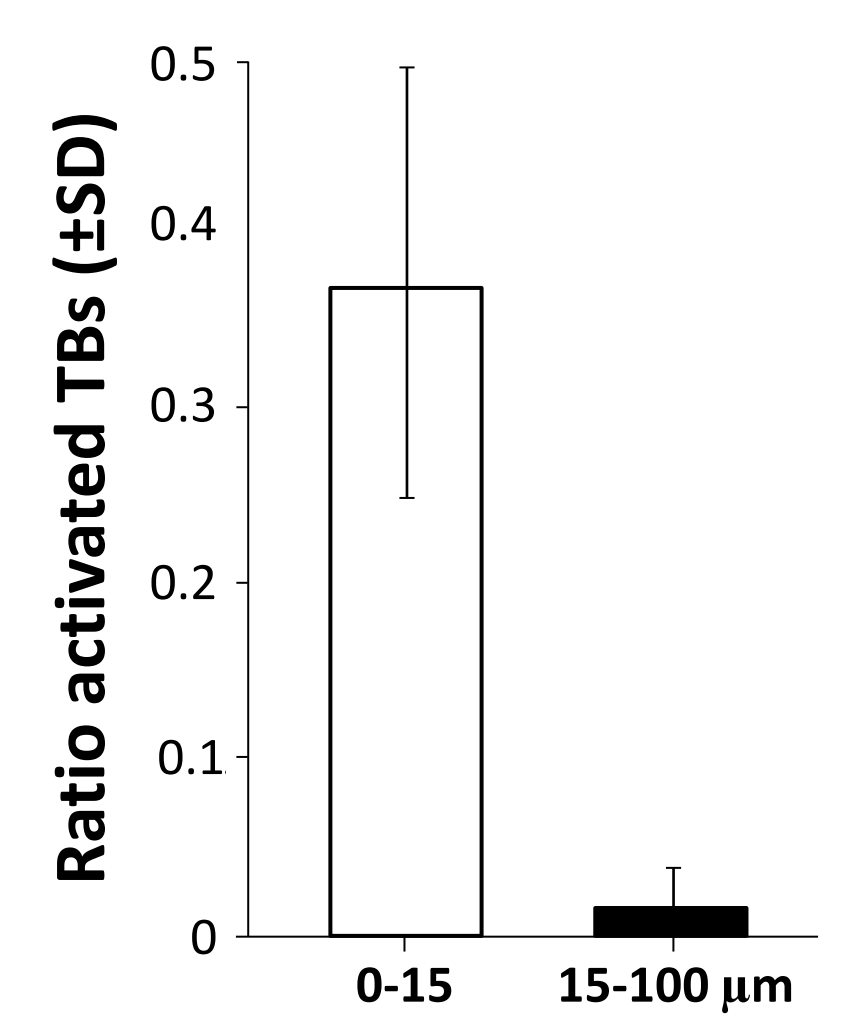
(2) Stylet sheath in tissue



(3) Aphid-infested tissue immunostained for P2



(4) Aphid-infested tissue immunostained for P4 (viral particles)

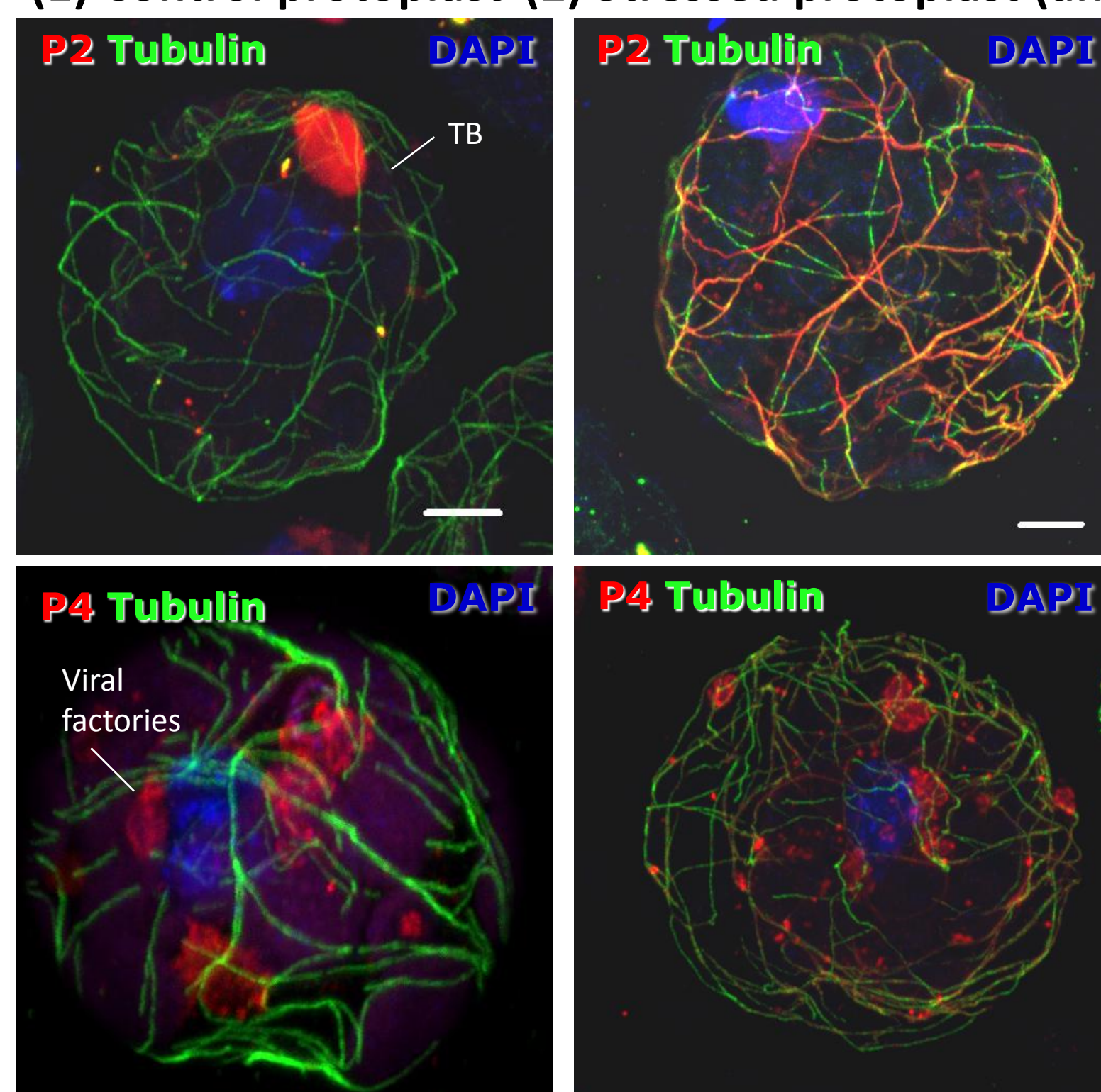


(5) Fraction of activated TBs at short and long distance from stylet sheaths

Immunofluorescence microscopy was used to detect the TB phenotype in non aphid-infested tissues (1) and in aphid-infested tissues (3,4). To identify TBs having been in contact with aphids, we tracked by autofluorescence the stylet sheaths that the aphids leave behind in the tissue (2). Approximately 35% of the TBs close to the stylet sheaths are activated (5).

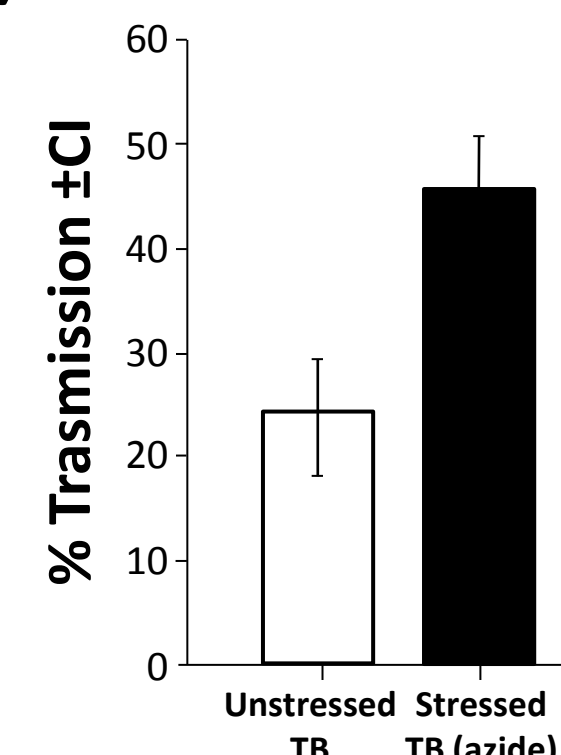
### Identification of stresses, which activate the TBs

(1) Control protoplast (2) Stressed protoplast (anoxia)

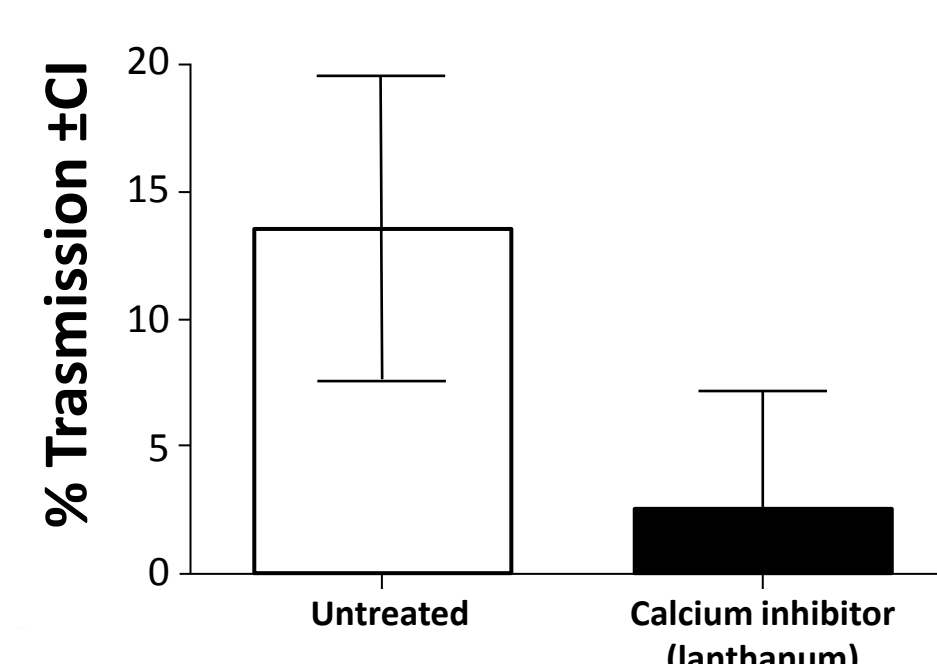


Type of treatment	Treatment	Effect on TB
Hormones	Abscisic acid	-
	Jasmonate	-
	Auxin	-
	Salicylic acid	-
	Electroporation	-
	Heat shock	+
	Membrane depolarisation	-
	Membrane hyperpolarisation	-
	Microwaves	-
	Ultrasonication	-
Elicitors	Vortexing	-
	Light/dark cycle	-
	UV	-
	Trinitrophenol	-
	Arabinogalactan	-
	Chitosan	-
Others	Cryptogel	-
	Sodium azide	+
	Anoxia	+
	Osmotic stress	-
	pH	-

(3) Different stresses induced or not the TB disruption



(4) Virus transmission rate with or without stress treatment



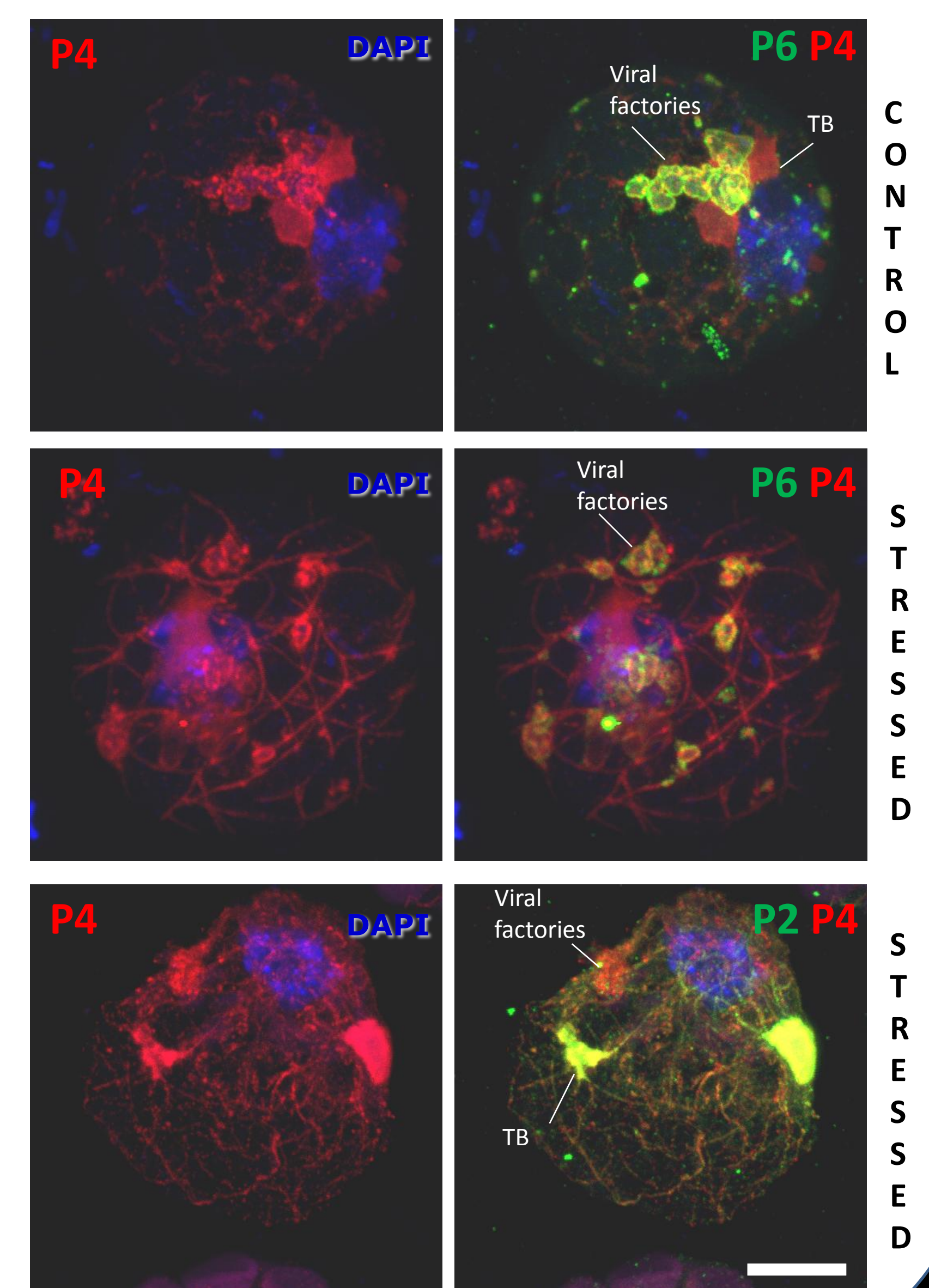
(5) Virus transmission rate with or without calcium drug treatment

The table(3) represents different stresses tested which induced or not the TB activation. Immunofluorescence microscopy was used to detect the TB phenotype in control protoplasts (1) and in stressed protoplasts (2). Stress-induced TB activation correlated with increased virus transmission rates (4). On the other hand, calcium channel blockers inhibited virus transmission suggesting a role of calcium in the TB activation (5).

### Origin of viral particles redistributed on microtubules

We used immunofluorescence microscopy to investigate the origin of the viral particles in stressed protoplasts. P6, P4 and P2 immunostaining identified viral factories, viral particles and TBs, respectively.

The results suggest that viral particles derive from TBs and viral factories.



## DISCUSSION

CaMV seems to « sense » its aphid vector and immediately prepares its transmission by the redistribution of P2 and the viral particles throughout the cell via the microtubule network. Only some specific stresses trigger the TB activation. This will allow us to define more precisely the mechanisms of TB activation. Calcium signalling pathways seem to be involved in this phenomenon. Another interesting question is: How is P2 transported on microtubules?