The plant pathogen *Agrobacterium tumefaciens* and the plant symbiont *Bradyrhizobium japonicum* produce the typical eukaryotic membrane lipid phosphatidylcholine (PC), which is required for efficient interaction with their host plants. In one of the two distinct pathways phospholipid N-methyltransferases (Pmts) methylate phosphatidylethanolamine (PE) via three successive reactions producing the intermediates monomethyl-PE (MMPE) and dimethyl-PE (DMPE) and finally PC. Bacterial Pmts are classified into *Sinorhizobium*-type (S-Pmt) and the *Rhodobacter*-type (R-Pmt) enzymes. PmtA (S-type Pmt) from *A. tumefaciens* catalyzes all three methylations of PE to yield PC. In contrast, *B. japonicum* requires two Pmt enzymes for PC formation. Whereas PmtA (S-type Pmt) catalyzes the formation of MMPE, PmtX1 (R-type Pmt) uses MMPE to form DMPE and PC. Bacterial Pmt enzymes are peripheral membrane proteins interacting transiently with the cytoplasmic membrane. Regulation of membrane to cytosol cycling is poorly understood and the lipid-contact sites are unknown. The aim of this work was to identify membrane-binding regions and to characterize membrane-binding mechanism(s) of *A. tumefaciens* PmtA and *B. japonicum* PmtA and PmtX1, which served as model enzymes for the two distinct Pmt types.

*A. tumefaciens* PmtA attaches the membrane via electrostatic interactions with anionic lipids in the membrane. This interaction is further stabilized by a hydrophobic insertion into the membrane core. At high PC concentrations, PmtA dissociates from the membrane. Since binding of PmtA to liposomes depends on the liposome size, PmtA seems to respond to membrane curvature. Two predicted amphipathic α-helical regions contribute to membrane binding. The N-terminal helix αA seems to be predominantly responsible for membrane recruitment and is crucial for enzyme activity. Membrane attachment of αA is mediated by a membrane-binding motif composed of two basic residues and a conserved hydrophobic phenylalanine (F19). In contrast to αA, the αF region folds into a helix upon association with anionic lipids, which might promote a conformational change in PmtA. A combination of transmission electron microscopy analysis and *in vitro* liposome-interaction studies revealed a membrane-remodeling activity of *A. tumefaciens* PmtA independent of
its enzymatic activity. Membrane deformation into vesicles or tubules strictly depends on
the lipid composition. Whereas the anionic lipid cardiolipin (CL) is essential for both
tubulation and vesiculation, MMPE or DMPE promote vesiculation activity of PmtA. The αA
region of PmtA is necessary and sufficient for liposome remodeling. The hydrophobic
residues F20 and V24 in the predicted hydrophobic face of αA are required for membrane
deformation. Presumably, membrane remodeling is based on shallow hydrophobic
insertion of the hydrophobic face into the lipid bilayer.

Similar to A. tumefaciens PmtA, B. japonicum PmtA and PmtX1 contain amphipathic αA
and αF regions. αA and αF of B. japonicum PmtA and αA of PmtX1 function as membrane-
contact sites. Analogous to the αA region of A. tumefaciens PmtA, αA of B. japonicum
PmtA is able to deform model membranes suggesting a conserved function as membrane-
attachment and -remodeling site in Pmts. Like A. tumefaciens PmtA, B. japonicum PmtA
might be recruited to the inner leaflet of the cytoplasmic membrane via ionic interactions
since αA binds anionic lipids via the basic residues R14 and K28.

Taken together, the present work provides first insights into the membrane-binding
mechanism of bacterial Pmt enzymes. Another important aspect is the discovery of a
membrane-deformation activity of Pmt enzymes opening new avenues for further
research on these enzymes.