6 SUMMARY

TraG/VirD4-like proteins are essential components of bacterial type IV secretion systems. During secretion, they are thought to translocate defined substrates through the inner cell membrane. The energy for this transport is presumably delivered by hydrolysis of nucleotides, since sequence-analysis identified TraG/VirD4-like proteins as potential nucleotide hydrolases (NTPases). In the present work, four representatives of the family of TraG/VirD4-like proteins were biochemically characterized and partly genetically analyzed: TraG (RP4), TrwB (R388), TraD (F) and HP0524 (*H. pylori*). These proteins were found to have a pronounced tendency to form oligomers and to bind DNA without sequence specificity. The purified proteins did not possess the proposed NTP hydrolyzing activity *in vitro*. TraG was however shown to bind ATP, as it had previously been detected for TrwB. Apart from binding ATP, TraG and TrwB were also found to bind ADP. Other nucleotides (GTP, CTP, UTP, dTTP) were effective competitors for ATP-binding. The DNA- and nucleotide-binding activities were both inhibited by Mg^{2+} and were found to functionally overlap. Using surface plasmon resonance (SPR) technology, the interaction of TraG with the essential secretion component TraI was demonstrated, providing the first direct evidence for this type of interaction. Topology analysis of TraG revealed that TraG contains a membrane anchor close to the N-terminus. Functionally essential domains of TraG were identified by insertion mutagenesis. The identified sequence domains were mapped to the corresponding structural determinants by comparison to the known crystal structure of TrwB, providing a detailed overview on the relation between sequence, structure, and function of TraG-like proteins. Deletion- and point mutation derivatives of TraG and TrwB were purified and characterized. The cytoplasmic domain was found to be functional in both DNA- and ATP-binding. However, removal of the membrane anchor prevented oligomerization of TraG and TrwB. Furthermore, the affinity of TraG for TraI was lost, probably as a direct consequence of the failure of the truncated protein to oligomerize. Point mutation at conserved Walker A sequence motif (P-loop motif) of TraG caused a significant decrease of its nucleotide-binding activity but did not affect the affinity for DNA or TraI. In this study, the multiple activities of TraG and TrwB were thus dissected structurally and functionally. Comparative analysis of several representatives of the family of TraG/VirD4-like proteins contributed substantially in understanding the function of this key component of type IV secretion.