6 APPENDIX I

6.1 Valuation of a putative TAP transmembrane topology

To this date crystal structures of three ABC transporters exist, those of the lipid A exporter MsbA of *E. coli* (Chang and Roth, 2001) and *Vibrio cholerae* (Chang, 2003) and of the vitamin B_{12} importer BtuCD of *E. coli* (Locher et al., 2002). So far, the transmembrane topology of TAP has not been solved and different models of the translocation pore exist (see Introduction 1.3.2). In the model by Vos and coworkers 8 TMSs were suggested for TAP1 (experimental approach) (Vos et al., 1999), whereas Abele and Tampe suggested 10 TMSs (sequence alignments to the P-gp MDR transporter) (Abele and Tampe, 1999). Here the TAP sequence is analyzed in comparison to the known transmembrane topology of MsbA. Sequence similarities between the TMDs of different ABC transporters can be found only if they transport related substrates in the same direction (Saurin et al., 1999). The TMSs of the halftransporters MsbA and BtuCD were shown to diverge significantly in their positions. However, MsbA and BtuCD are ABC transporters of different types; MsbA exports its substrate, whereas BtuCD imports it with the help of a binding protein (BtuF). In addition, BtuCD consist of 20 (2x10) TMS in contrary to only 12 (2x6 TMS) for MsbA. Of these, the MsbA transporter is closest related to TAP, in fact, it has one of the highest homology (28% identical to TAP1 and TAP2 on protein level, Fig. 6.2) to TAP among other ABC transporters, only the P-gp transporter of the P-gp transporter is closer related to TAP (35% identity), whereas the TMD of BtuCD shows no significant similarity to TAP. The predicted transmembrane topology of VC-MsbA (NP_231512) and human TAP1 (CAA40741) by Argos and von Heyne results in strikingly similar diagrams (Fig. 6.3). Only the two last TMSs of TAP1 are not as distinct as for MsbA. Furthermore, the sequence alignment of MsbA and TAP1 shows similar pattern and length of lumenal and cytosolic loops (Fig. 6.2). The crystal structure of *V. cholerae* and *E. coli* MsbA revealed the positions of the TMSs (TMS1-TMS6) in relation to each other (Fig. 6.1). The first extracellular (on the top side of the TMD, opposite to the cytosolic NBDs) loop of MsbA is long and reaches from one side of the protein to the other, from TMS1 to TMS2. TMS3 and

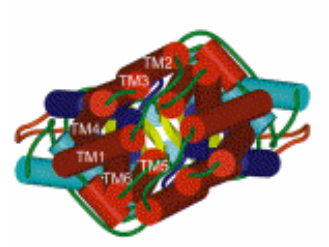

Fig. 6.1 Top view of VC-MsbA.
Extracellular connecting loops are shown in green, TMSs as red cylinders (Chang, 2003).

Fig. 6.2 Amino acid sequence alignment of VC-MsbA and human TAP1.

Amino acid sequence of the TMD of VC-MsbA and TMS5-10 of TAP1 are shown. Identical amino acids are highlighted in purple and similar in red. TMS1-6 of VC-MsbA (Chang, 2003) are marked with a black dotted line, whereas predicted TMS5-10 of TAP1 (Abele and Tampe, 1999) are marked with a grey dotted line.

TMS4 are positioned next to each other with only a short loop in the extracellular space connecting them. Forming the other side and facing the bilayer, TMS5 and TMS6 are in close vicinity connected by a short extracellular loop. When examining the sequence alignment of MsbA and TAP1 it is noticeable that the extracellular and lumenal loops of MsbA and TAP1 respectively, have the same length between the corresponding TMSs. Taken together, the high homology between TAP1 and MsbA, analogue predicted transmembrane topology and similar pattern and length of loops between the TMS1-6 of MsbA and the predicted TMS5-10 of TAP1 point at a possibly similar organisation of the TAP TMD as of the MsbA.

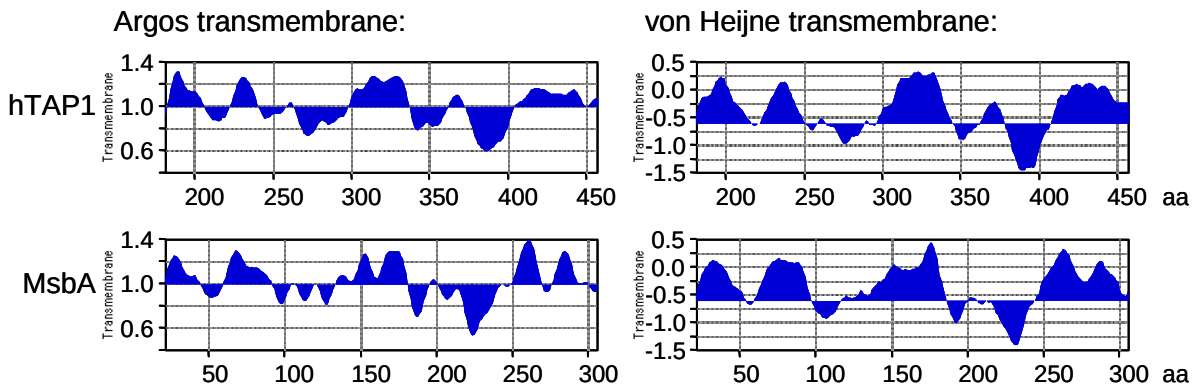

Fig. 6.3 VC-MsbA and human TAP1 prediction of transmembrane topology.

Amino acid sequence of the TMD of VC-MsbA and TMS5-10 of TAP1 plotted by Argos and van Heijne transmembrane prediction parameters.

SUMMARY

- **Prediction of transmembrane topology of TAP1 and MsbA results in similar transmembrane pattern.**
- **Corresponding loops of MsbA and TAP1 present the same length and close homology.**
- **Transmembrane topology of TAP similar to MsbA ?**