Günther Winkelmann (Ed.)

Microbial Transport Systems

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Preface

"Please, pass the salt" is something that could be asked by microorganisms as well as gourmets. How do cells transport nutrients? An essential feature of all living organisms is the ability to accumulate nutrients against a concentration gradient and to excrete the various end products of metabolism. The topic of microbial transport systems involves a variety of other issues, such as generation of a membrane potential, homeostasis of ions, maintaining an osmotic balance, excretion of enzymes and toxins, the release of hormones and signals, drug resistance strategies, etc. The main cellular structure responsible for nutrient transport is the plasma membrane, which may be accompanied by an outer membrane in the case of gram-negative bacteria. Due to their long evolutionary development, microbial cells are the most diverse with respect to transport. The various mechanisms of solute transport across these membranes are so diverse that it is surprising that cells can manage the traffic of so many different compounds simultaneously. Cells obviously avoid traffic jams by two principal mechanisms, that is by up- or down-regulation and by energetic activation and inactivation of transporters and channels. Although a distinction between primary transporters (F-type ATPase, P-type ATPase, ABC-ATPase), secondary transporters (major facilitators, channels) and group translocation is generally made, many more strategies occur. While channel-type facilitated diffusion is common among pore-forming compounds, active transport against a concentration gradient occurs via ABC transporters, P-type ATPases, MFS transporters and group translocation. While some of these use direct ATP hydrolysis for transport, MFS transporters use indirect energy from a membrane potential, which in turn connects ion gradient to solute flow resulting in uniport, symport and antiport mechanisms.

This diversity of transport systems has necessitated the development of a transporter classification (TC) system (see Chapter 1 of Milton Saier).

It is the aim of the present book to demonstrate how some important nutrients are transported into the cells, how proteins are excreted and how the diverse transport mechanisms operate. Gene replacing techniques of transport genes, hydropathy plots, mutational analysis and structural and functional genomics are modern tools in transport biology which have led to unraveling the secrets of transport mechanisms. Although this book cannot be comprehensive it should inspire and

encourage further studies. Including every topic on transport would generate a book three times this length and far too expensive – therefore, I hope to have selected the essentials.

My thanks go to all authors for their willingness to participate in this project and for producing their manuscripts so promptly. I am especially grateful to Carl J. Carrano, Volkmar Braun, Klaus Hantke, Dick van der Helm and Milton Saier for helpful suggestions and comments.

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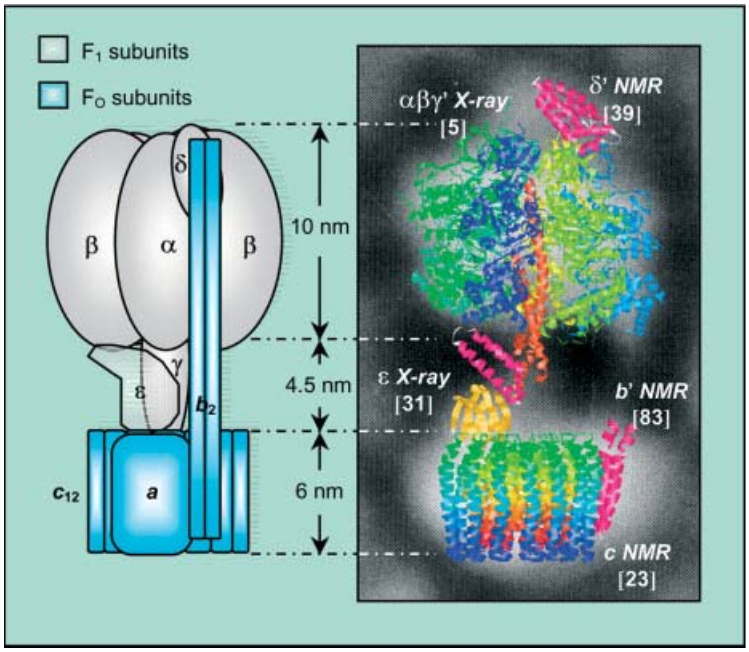
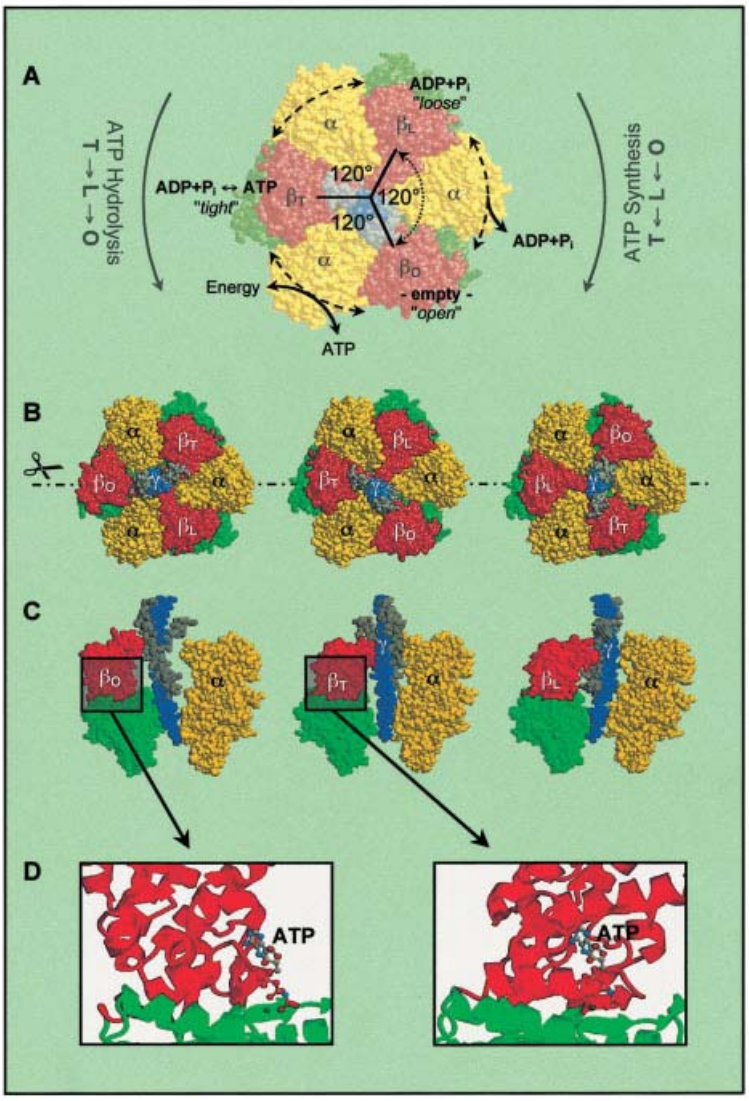
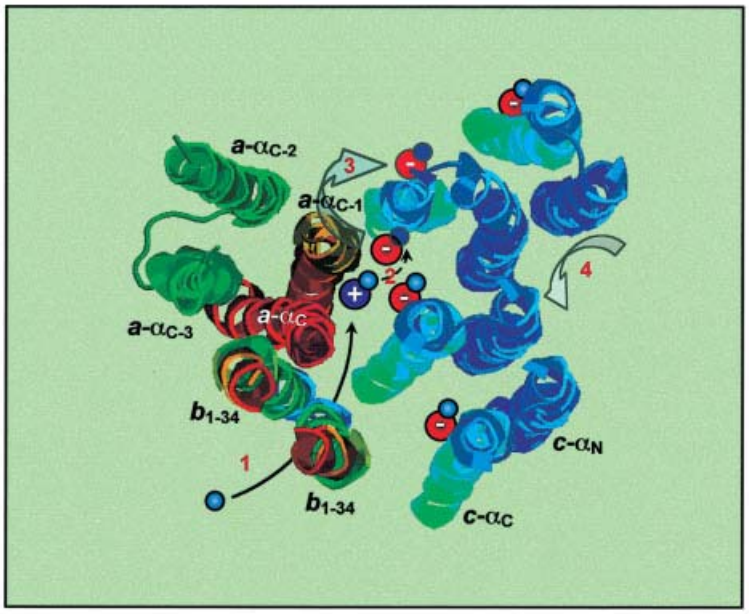
Color Plates



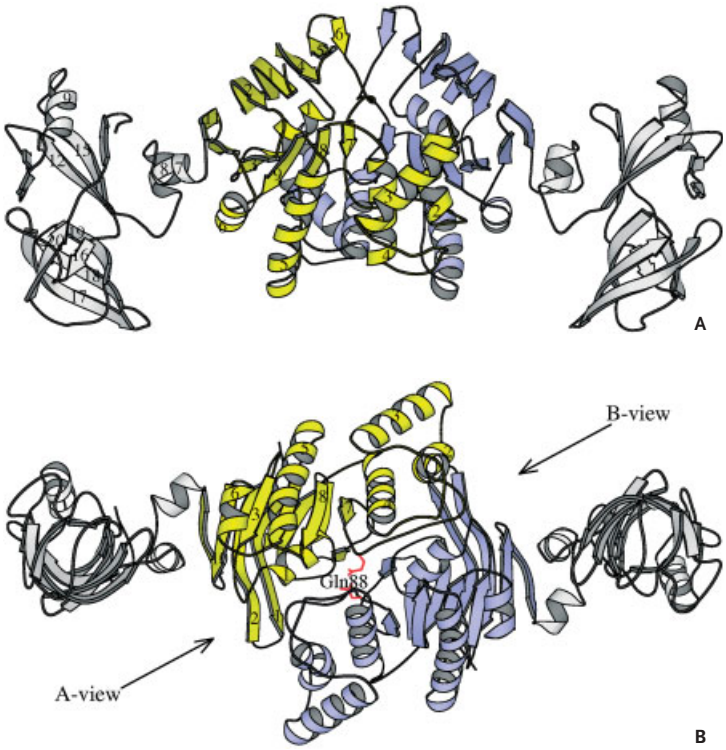
Chapter 2, Fig. 1. Schematic presentation of the F_1F_O ATP synthase. Overview of subunit assembly and modeling of available structural information from either NMR spectroscopy or X-ray crystallographic analysis into the electron density map of the *E. coli* F_1F_O complex (taken from [7] with kind permission from *Nature*). Corresponding references are quoted in brackets.


A
ATP Hydrolysis
T → L → O
ADP+Pi ↔ ATP
"tight"
ADP+Pi
"loose"
120°
120°
120°
ADP+Pi
ATP Synthesis
T ← L ← O
Energy
- empty -
"open"
ATP
B
C
D
ATP
ATP

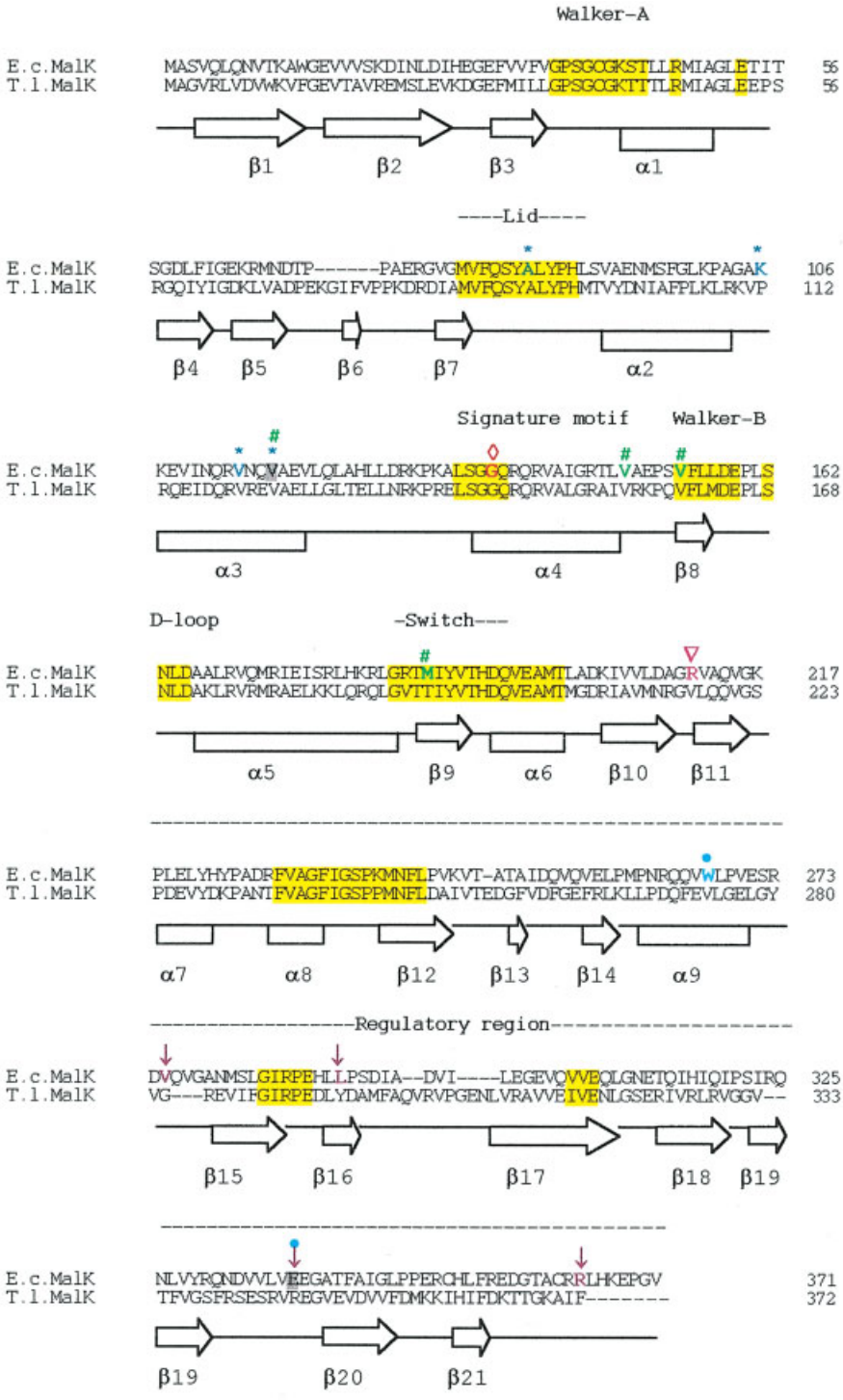
◀ **Chapter 2, Fig. 2.** Catalysis within the F_1 complex – the binding change mechanism. **A** Different conformations assumed sequentially by each catalytic site during synthesis or hydrolysis of ATP as subunit γ rotates 120° within the $\alpha_3\beta_3$ hexamer. Sites are designated as "open" (β_O, no nucleotide bound), "loose" (β_L, ADP+ P_i bound), and "tight" (β_T, interconversion of bound ADP + P_i and ATP). The sketch of the crystal structure from the bovine heart F_1 complex [5] is depicted as seen from the membrane. Clockwise rotation of subunit γ leads to ATP synthesis, whereas counter-clockwise rotation corresponds to ATP hydrolysis. Based on kinetic data it is likely that during steady state catalysis the "open" site is immediately occupied by another nucleotide. **B** Circulating conformational changes within the $\alpha_3\beta_3$ hexamer as subunit γ rotates stepwise at intervals of 120° each in counter-clockwise direction (i.e., ATP hydrolysis). **C** Cross-section through **B**. Nucleotide-dependent conformational changes within the C-terminal domain of the β-subunit during subunit γ rotation. Whereas the C-terminal domain undergoes spatio-temporal rearrangements during the catalytic cycle (red color), the N-terminal portion of subunit β (green) retains an approximately threefold symmetry around the rotational axis. The N- and C-terminal domain of subunit γ is depicted in gray and blue, respectively. **D** Clipping of the subunit β hinge region in either "open" (left) or "tight" (right) conformation. Refer to Sect. 4 for further details. Molecular sketches are kindly provided by Dr. G. Oster (Copyright © 2001, University of California, Berkeley).



Chapter 2, Fig. 5. Hand-over-hand pattern of the proton translocation pathway within the assembled F_O complex. Structural sketches are shown from four of the *c*-subunits (both the N- and C-terminal helix, *c*-α_N and *c*-α_C, respectively) as well as from the transmembrane domain of the subunit *b* dimer ($b_{1\text{-}34}$) and from the four C-terminal helices of subunit *a* (*a*-α_C – *a*-$\alpha_{C\text{-}3}$) according to [92]. The assembly is presented as seen from the F_1 complex. The proposed functional cycle for the translocation of one proton is depicted according to the two-channel model established for the *E. coli* ATP synthase. The proton enters the complex via the inlet channel from the periplasmic side of the membrane, involving the positive stator charge *a*R210 (1). In the resting state, residue *a*R210 is sandwiched by both a protonated and a deprotonated *c*D61 side chain at the periphery of the subunit *c* oligomer. After proton transfer to *c*D61 (2), the C-terminal helix of the newly protonated monomer rotates 140° in order to adopt its protonated orientation (3), resulting in a fully protonated intermediate state of the oligomer. Simultaneously, by the interaction of *c*D61 and *a*R210 during helix rotation, the subunit *c* ring is pushed to rotate contrarily one step ahead (4), placing residue *a*R210 at the interface of the subsequent set of neighboring *c*-subunits. Concomitantly, residue *c*D61 of the next *c*-subunit loses its proton to the cytoplasmic side via the outlet channel (not shown), accompanied by rotation of the C-terminal helix in order to regenerate the deprotonated conformation of the resting state.



Chapter 4, Fig. 5. Ribbon representation of the *Thermococcus litoralis* MalK dimer.The A- and B-molecules are colored yellow and blue, respectively, except for both regulatory domains which are gray. Labels indicate numbers of strands and helices according to the secondary structure assignment given in Fig. 6. **(A)** The side view shows the extended dumbbell shape resulting from the two regulatory domains on either end and the central ATPase domain dimer. The pseudo-twofold symmetry axis is oriented vertically and runs through the center of the dimer. The strong involvement of helices 2 and 4 in dimerization is seen. The bottom part of the dimer is supposed to interact with the TMDs MalFG. **(B)** The bottom view along the pseudo-twofold axis shows the deviation from twofold symmetry. The helical layer of one monomer is seen in contact with the two upper layers containing the nucleotide binding site of the other monomer. The symmetry axis between strands 6 of both monomers seems to provide a mechanical hinge for the dimer. Residues Gln88 from both monomers are shown to demonstrate their close apposition. The A- and B-viewing directions are indicated. Taken from [31] with permission from the author and the publisher.


Walker-A
E.c.MalK MASVQLQNVTKAWGEVVVSKDINLDIHEGEFVVFVGPSGCGKSTLLRMIAGLETIT 56
T.l.MalK MAGVRLVDVWKVFGEVTAVREMSLEVKDGEFMILLGPSGCGKTTTLRMIAGLEEPS 56
β1 β2 β3 α1
----Lid----
E.c.MalK SGDLFIGEKRMNDTP------PAERGVGMVFQSYALYPHLSVAENMSFGLKPAGAK 106
T.l.MalK RGQIYIGDKLVADPEKGIFVPPKDRDIAMVFQSYALYPHMTVYDNIAFPLKLRKVP 112
β4 β5 β6 β7 α2
Signature motif Walker-B
E.c.MalK KEVINQRVNQVAEVLQLAHLLDRKPKALSGGQRQRVAIGRTLVAEPSVFLLDEPLS 162
T.l.MalK RQEIDQRVREVAELLGLTELLNRKPRELSGGQRQRVALGRAIVRKPQVFLMDEPLS 168
α3 α4 β8
D-loop -Switch---
E.c.MalK NLDAALRVQMRIEISRLHKRLGRTMIYVTHDQVEAMTLADKIVVLDAGRVAQVGK 217
T.l.MalK NLDAKLRVRMRAELKKLQRQLGVTTIYVTHDQVEAMTMGDRIAVMNRGVLQQVGS 223
α5 β9 α6 β10 β11
E.c.MalK PLELYHYPADRFVAGFIGSPKMNFLPVKVT-ATAIDQVQVELPMPNRQQVWLPVESR 273
T.l.MalK PDEVYDKPANIFVAGFIGSPPMNFLDAIVTEDGFVDFGEFRLKLLPDQFEVLGELGY 280
α7 α8 β12 β13 β14 α9
----------Regulatory region----------
E.c.MalK DVQVGANMSLGIRPEHLLPSDIA--DVI----LEGEVQVVEQLGNETQIHIQIPSIRQ 325
T.l.MalK VG---REVIFGIRPEDLYDAMFAQVRVPGENLVRAVVEIVENLGSERIVRLRVGGV-- 333
β15 β16 β17 β18 β19
E.c.MalK NLVYRQNDVVLVEEGATFAIGLPPERCHLFREDGTACRRLHKEPGV 371
T.l.MalK TFVGSFRSESRVREGVEVDVVFDMKKIHIFDKTTGKAIF------- 372
β19 β20 β21