Wolfgang Schumann

Dynamics of the Bacterial Chromosome

Structure and Function

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Structure and Function

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Cover illustration:
The picture shows DAPI (blue) and FM4-64-stained (red) pre-divisional sporangia of *Bacillus subtilis* of a RacA-GFP-producing strain at h 1.5 of sporulation. RacA-GFP (green) is seen as foci at the poles and as a haze over the nucleoids. From the work of S. Ben-Yehuda, D. Rudner and R. Losick **2003**, Science 299, 532.

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Contents

Foreword

Over the past 50 years, molecular microbiology has provided fundamental contributions not only to the understanding of microbiology as a unique discipline but also to the understanding of what makes a living cell. In 1945 Schrödinger in his famous lecture ''What is life?'' addressed the challenging task of defining the properties of a necessary (e.g. minimal/required) information-containing system. More than half a century later, we can state that many aspects of this fundamental question ''What is life?'' have been answered. This series of great discoveries started with the elucidation of the DNA structure by F. Crick and J. Watson more than 50 years ago and was subsequently expanded by the exploration of the basic mechanisms of DNA replication and gene expression. These scientific achievements were complemented and fostered by the development of crucial techniques, such as gene cloning, DNA sequencing and PCR. The discoveries culminated in an event that will certainly have a fixed position in future text books of science history: the first complete elucidation of a genome sequence of a living organism, *Haemophilus influenzae*, in 1995. At the beginning of 2006, the complete genomes of nearly 300 bacterial species are available. Knowledge of the complete genome sequence is an essential prerequisite in order to gain a comprehensive understanding of the molecular mechanisms of life. The genome sequence, however, only provides the ''blueprint'' of life, not life itself. Now, functional genomics such as transcriptomics, proteomics, metabolomics and bioinformatics are required to bring the blueprint of life to the real life of living organisms. The combination of the expertise and accumulated knowledge in the traditional disciplines such as microbiology, biochemistry, molecular genetics and molecular biology with this panoramic view of the recent ''omics technologies'' on the cell as an entity will enable/facilitate a new quality in the understanding of what makes a cell viable. Because of their low complexity, single-cell bacterial systems such as *Escherichia coli*, *Bacillus subtilis* and others constitute perfect model systems for tackling such an ambitious goal as understanding life as an entity. The unexpected finding that, even in the best analyzed model organisms such as *Escherichia coli*, one-third of all genes codes for proteins with still unknown functions emphasizes the challenge and indicates that many pages of the ''bible of life'' are still empty.

There is a comprehensive literature on the entire field of molecular microbiology, from the structure, flexibility and stability of bacterial genomes to the various facets of the regulation of gene expression, a field that even in bacteria includes mechan-

isms of not only transcriptional initiation but also transcriptional elongation and termination and regulation at the posttranscriptional, translational and posttranslational level. Even if the first description of the regulation of a bacterial operon, the *lac*-operon in *E. coli* by Jacob and Monod, was a milestone in the history of microbiology, honored by the Nobel Price in 1965, we had however to realize that this regulation mechanism represents only one of hundreds of mechanisms evolved during the three billion years that bacteria have populated our planet. For those involved in teaching students, it is difficult to keep up with the pace of the development of all these different fields of molecular microbiology. A textbook covering all these aspects of molecular microbiology is equally useful for teachers and students in microbiology at universities, because this very genuine, innovative and essential new field of microbiology needs more space in the teaching programs for life science students.

Wolfgang Schumann, professor for genetics at the University Bayreuth, accepted the challenge of writing a textbook on molecular bacteriology. For many years, he has lectured students of biology and biochemistry on the genetics and molecular biology of bacteria. He is appreciated as an expert in molecular genetics and bacterial gene regulation and his contributions to many fields of molecular genetics, e.g. the mechanisms of heat induction in Gram-positive bacteria and the discovery of the CIRCE element (to mention only some of them), are accepted worldwide.

I have always been impressed by his detailed knowledge of the scientific literature on quite different fields of molecular microbiology. This is one reason which convinced me that Wolfgang Schumann is the right person to take over this ambitious task. I have already used the chance to read some of the chapters of this book in preparing my lectures at the University of Greifswald this year. I enjoyed this reading, very conveniently providing me with an excellent survey. Assessing the recent literature critically and in detail an essential part of the training of young students has been done in an excellent way. All essential aspects of molecular bacteriology (which might be extended by a similar textbook by other experts on the molecular microbiology of Archaea or eukaryotic microorganisms) have been addressed in a very authentic, comprehensive manner and have been illustrated by a lot of impressive and useful figures.

This book covers the most essential chapters in molecular microbiology, starting with the structure of the bacterial cell via the organization of bacterial chromosomes to the bacterial cell cycle, followed by chapters on molecular genetics, recombination and mutations and their repair. A comprehensive chapter deals with the various mechanisms of gene regulation, followed by a description of the role of chaperones in protein quality control and protein secretion mechanisms. An extra chapter on stress genes and their regulation is a reflection of the specific research interests of the author. A final chapter on gene transfer makes the book complete. I just missed a chapter on the prokaryotic development of bacteria, including endospore-forming bacteria such as *B. subtilis*, heterocyst formation in *Anabaena* and the dimorphic life cycle of *Caulobacter crescentus*, to mention some.

All in all, this new book offers an actual, comprehensive view of the present state of the art in the field of molecular bacteriology. It is a very valuable source of information not only for students, but also for scientists who wish to become acquainted with one of the most exciting fields of current microbiology.

Greifswald, 04.04.2006 Michael Hecker

Bacterial Species and their Abbreviations

A. actinomycetemcomitans	*Actinobacillus actinomycetemcomitans*
A. salmonicida	*Aeromonas salmonicida*
A. tumefaciens	*Agrobacterium tumefaciens*
A. vinelandii	*Azotobacter vinelandii*
B. bronchiseptica	*Bordetella bronchiseptica*
B. burgdorferi	*Borrelia burgdorferi*
B. japonicum	*Bradyrhizobium japonicum*
B. megaterium	*Bacillus megaterium*
B. pertussis	*Bordetella pertussis*
B. polymyxa	*Bacillus polymyxa*
B. pseudomallei	*Burkholderia pseudomallei*
B. hyodysenteriae	*Brachyspira hyodysenteriae*
B. subtilis	*Bacillus subtilis*
C. acetobutylicum	*Clostridium acetobutylicum*
C. crescentus	*Caulobacter crescentus*
C. difficile	*Clostridium difficile*
C. diphtheriae	*Corynebacterium diphtheria*
C. freundii	*Citrobacter freundii*
C. jejuni	*Campylobacter jejuni*
C. perfingens	*Clostridium perfringens*
C. psittacii	*Chlamydia psittacii*
C. trachomatis	*Chlamydia trachomatis*
C. violaceum	*Chromobacterium violaceum*
D. radiodurans	*Deinococcus radiodurans*
E. amylovora	*Erwinia amylovora*
E. carotovora	*Erwinia carotovora*
E. cloacae	*Enterobacter cloacae*
E. coli	*Escherichia coli*
E. crysanthemie	*Erwinia crysanthemie*
E. faecalis	*Enterococcus faecalis*
H. ducreyi	*Haemophilus ducreyi*
H. halobium	*Halobacterium halobium*
H. influenzae	*Haemophilus influenzae*
H. pylori	*Helicobacter pylori*
H. salinarium	*Halobacterium salinarium*
K. oxytoca	*Klebsiella oxytoca*

K. pneumoniae	*Klebsiella pneumoniae*
L. lactis	*Lactococcus lactis*
L. monocytogenes	*Listeria monocytogenes*
M. genitalium	*Mycoplasma genitalium*
M. janashii	*Methanococcus janashii*
M. leprae	*Mycobacterium leprae*
M. loti	*Mesorhizobium loti*
M. pulmonis	*Mycoplasma pulmonis*
M. tuberculosis	*Mycobacterium tuberculosis*
M. xanthus	*Myxococcos xanthus*
N. gonorrhoeae	*Neisseria gonorrhoeae*
N. meningitis	*Neisseria meningitis*
P. aeruginosa	*Pseudomonas aeruginosa*
P. fluorescens	*Pseudomonas fluorescens*
P. gingivalis	*Porphyromonas gingivalis*
P. putida	*Pseudomonas putida*
P. syringae	*Pseudomonas syringae*
R. capsulatus	*Rhodobacter capsulatus*
R. etli	*Rhizobium etli*
R. solanacearum	*Ralstonia solanacearum*
S. agalactiae	*Streptococcus agalactiae*
S. albus	*Streptomyces albus*
S. ambofaciens	*Streptomyces ambofaciens*
S. aurantiaca	*Stigmatella aurantiaca*
S. aureus	*Staphylococcus aureus*
S. avermitilis	*Streptomyces avermitilis*
S. coelicor	*Streptomyces coelicor*
S. enterica	*Salmonella enterica*
S. flexneri	*Shigella flexneri*
S. hygroscopius	*Streptomyces hygroscopius*
S. meliloti	*Sinrhizobium meliloti*
S. pneumoniae	*Streptococcus pneumoniae*
S. pyogenes	*Streptococcus pyogenes*
S. sonnei	*Shigella sonnei*
S. tsukubaensis	*Streptomyces tsukubaensis*
S. typhi	*Salmonella typhi*
S. typhimurium	*Salmonella typhimurium*
T. aquaticus	*Thermus aquaticus*
T. maritima	*Thermotoga maritima*
T. thermophilus	*Thermus thermophilus*
V. alginolyticus	*Vibrio alginolyticus*
V. cholerae	*Vibrio cholerae*
V. fischeri	*Vibrio fischeri*
V. harveyi	*Vibrio harveyi*
X. campestris	*Xanthomonas campestris*
Y. enterocolitica	*Yersinia enterocolitica*
Y. pestis	*Yersinia pestis*

Color Plates

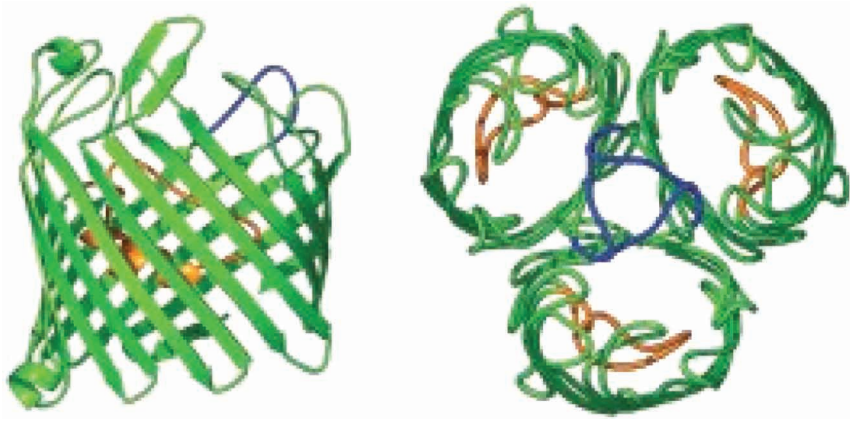

Fig. 1.2 Structure of the OmpF porin. View of the monomer from the side (left) and view of the trimer from the top. The loop inside the opening narrows the channel. H. Nikaido **2003**, *Microbiol. Mol. Biol. Rev.* 67, 593–556; Fig. 2.

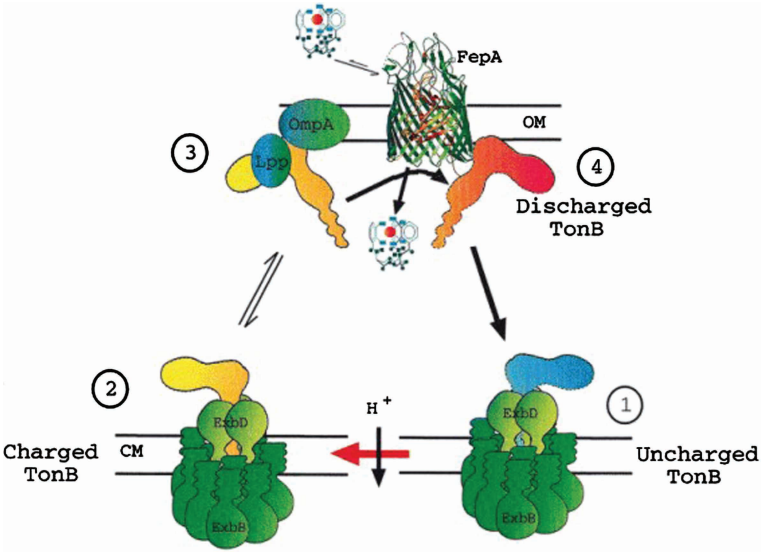

Fig. 1.3 Shuttle model for TonB-dependent energy transduction. Uncharged TonB (1) is energized by uptake of a proton (2) and shuttles to the outer membrane where is docks to OmpA/Lpp waiting for a transporter such as FepA with a bound ligand. Upon interaction with the Ton box of the the transporter the conformational energy is transduced to the transporter (3) triggering uptake of the ligand into the periplasm (4). The discharged TonB shuttles back to the ExbBD complex to become recharged. K. Postle, R.J. Kadner **2003**, *Mol. Microbiol.* 49, 869–882; Fig. 2.

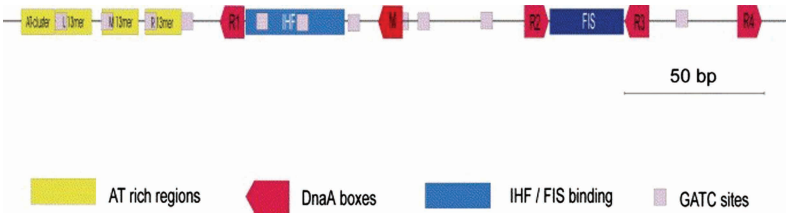

Fig. 3.1 Organization of the *oriC* region of *E. coli*. The *oriC* region of *E. coli* consists of five DnaA boxes called R1 through R4 and M, binding sites for the histone-like proteins FIS and IHF, three AT-rich regions and 11 GATC methylation sites. W. Messer **2002**, *FEMS Microbiol. Rev.* 26, 355; Fig. 1.

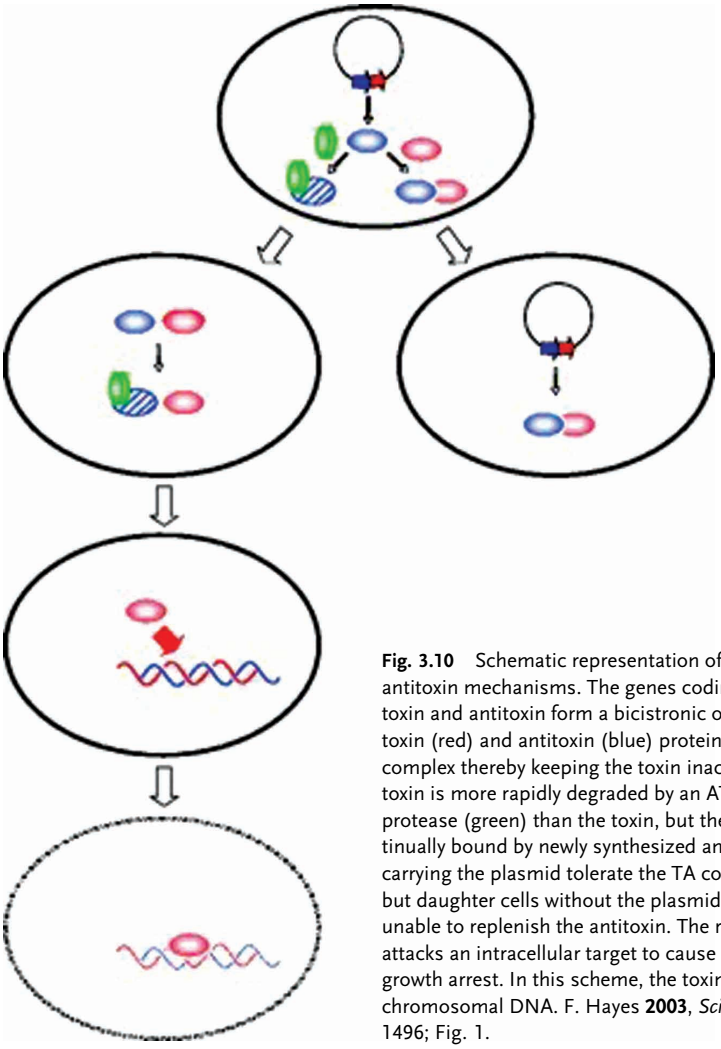

Fig. 3.10 Schematic representation of the toxin–antitoxin mechanisms. The genes coding for the toxin and antitoxin form a bicistronic operon. The toxin (red) and antitoxin (blue) proteins form a tight complex thereby keeping the toxin inactive. The antitoxin is more rapidly degraded by an ATP-dependent protease (green) than the toxin, but the latter is continually bound by newly synthesized antitoxin. Cells carrying the plasmid tolerate the TA complex (right), but daughter cells without the plasmid (left) are unable to replenish the antitoxin. The released toxin attacks an intracellular target to cause cell death or growth arrest. In this scheme, the toxin attacks the chromosomal DNA. F. Hayes **2003**, *Science* 301, 1496; Fig. 1.

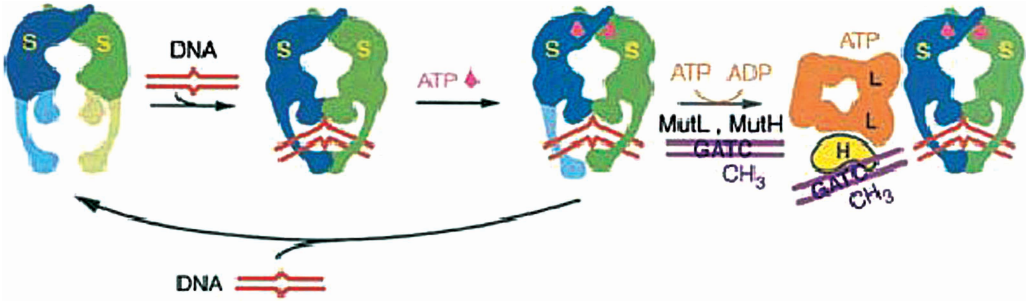

Fig. 5.2 Methyl-mediated mismatch repair. MutS recognizes and binds to the mismatch followed by binding of ATP. This complex recruits MutL which recognizes the nearest hemimethylated GATC and binds MutH. MutH cuts 5′ or 3′ to the unmodified GATC sequence, UvrD binds to the nick and unwinds the double-strand DNA followed by resynthesis and ligation. T.A. Kunkel, D.A. Erie **2005**, *Annu. Rev. Biochem.* 74, 681; Fig. 2C.

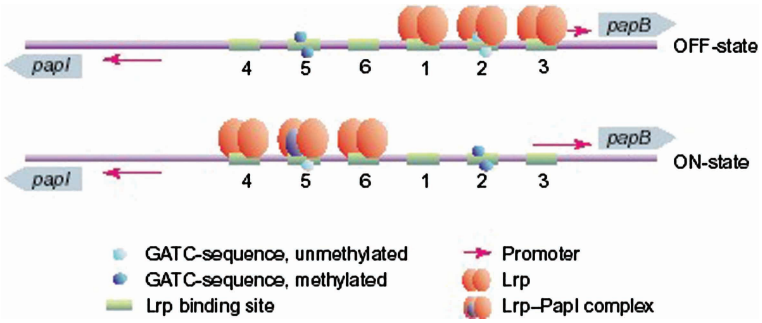

Fig. 6.3 Turning *pap* pilus synthesis on and off. See text for explanation who a cell can shift from the OFF- to the ON-state and vice versa. A. Løbner-Olesen, et al. **2005**, *Curr. Opin. Microbiol.* 8, 154–160.

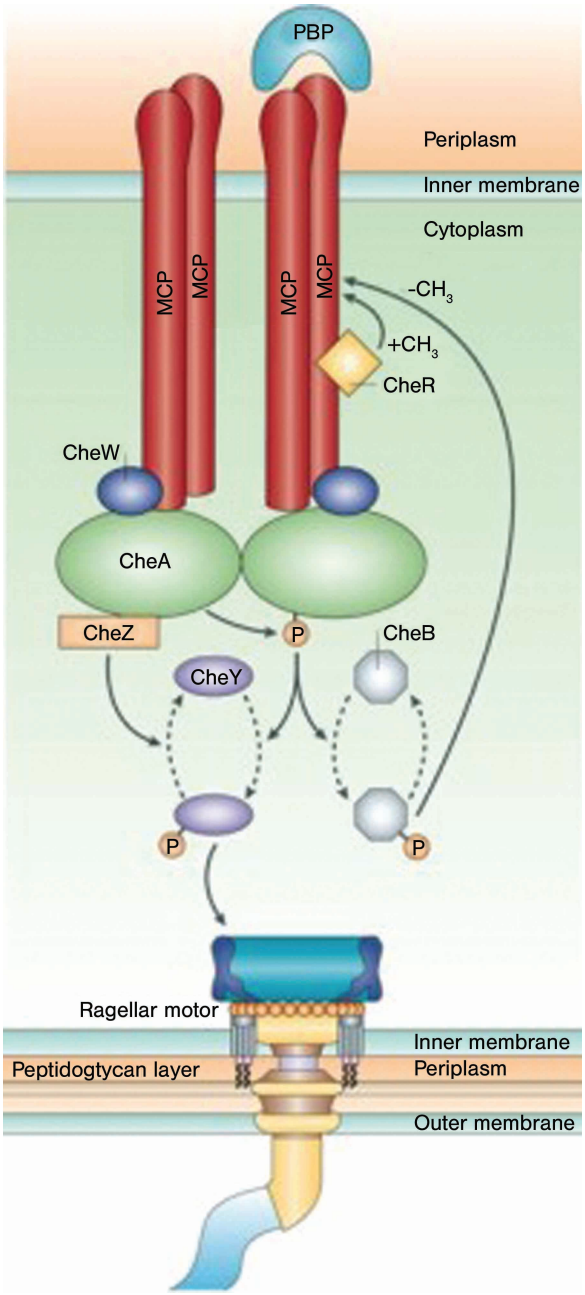

Fig. 6.27 Signalling pathways in bacterial chemotaxis. Dimeric MCPs form α-helical coiled-coil structures spanning the inner membrane. Chemical compounds (attractants and repellents) enter the periplasm through porins and interact either directly with the periplasmic domain of the appropriate chemoreceptor or indirectly through a *p*eriplasmatic *b*inding *p*rotein (PBP; MBP, RBP, GBP and DPP are *m*altose, *r*ibose, *g*alactose and *d*ipe*p*tide *b*inding *p*rotein, respectively). The CheW coupling molecule transduces the signals to the CheA sensor kinase which, after *trans*-phosphorylation, transfers the phosphate first to CheY and then to methylesterase CheB. CheY~P interacts with the flagellar motor to bring about a change in the direction. CheB~P competes with a constitutive methyltransferase, CheR, to control the degree of methylation of specific glutamates in the MCPs. This resets the signaling state of the chemoreceptors and allows them to adapt to the present concentration of attractant and to sense subsequent changes. Dephosphorylation of CheY~P is accelerated by the phosphatese CheZ. G.H. Wadhams **2004**, *Nat. Rev. Mol. Cell Biol.* 5, 1024–1037; Fig. 2.

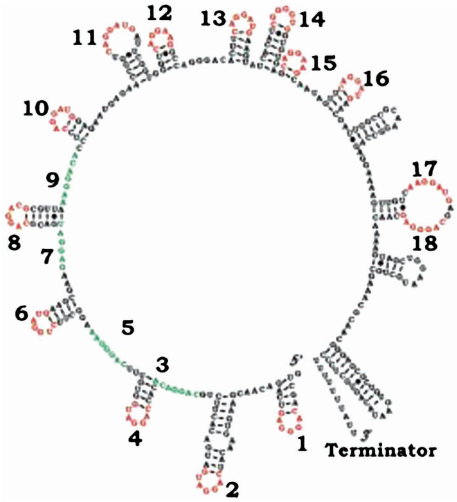

Fig. 6.37 Proposed structure of the CsrB RNA. This structure contains 18 repeated structures numbered 1 to 18 which may facilitate binding of CsrA. T. Romeo **1998**, *Mol. Microbiol.* 29, 1321–1330; Fig. 1.

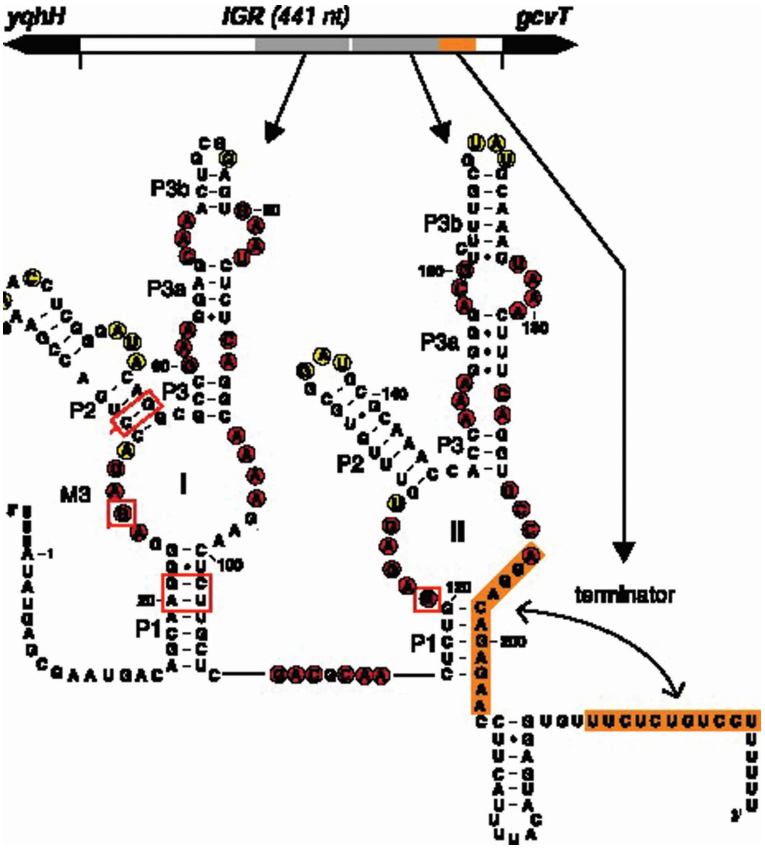

Fig. 6.38 The *B. subtilis gcvT* riboswitch. The intergenic region between *yqhH* and the *gcvPA-gcvPB* operon designated *gcvT* acts as a riboswitch and contains the two glycine sensing aptamers I and II. If the glycine concentration in the cell is low, the two aptamers remain unoccupied favoring formation of the terminator structure resulting in transcription attenuation. If the glycine concentration is high, it will bind to the two aptamers preventing formation of the transcription terminator, and the two structural genes will be expressed. M. Mandal, et al. **2004**, *Science* 306, 275; Fig. 4, modified.

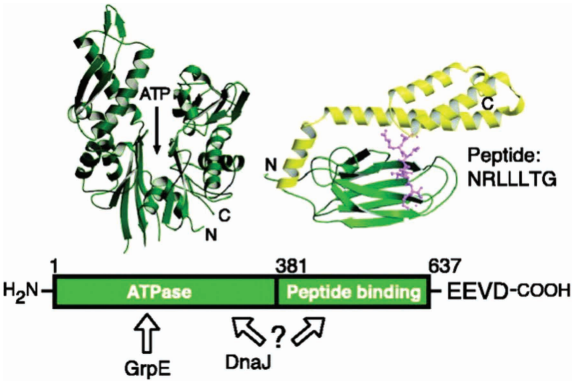

Fig. 7.1 Domain organization of the DnaK chaperone. DnaK consists of an N-terminal ATPase and a C-terminal peptide-binding domain. The ATPase domain consists of two lobes which form a deep cleft serving as a binding pocket for ATP. The peptide-binding domain (here shown with a heptapeptide substrate) forms a β sandwich composed of two sheets of four strands each, followed by α helices spanning back over the sandwich. Further indicated by open arrows are interaction sites for GrpE and DnaJ. F.U. Hartl, M. Hayer-Hartl **2002**, *Science* 295, 1852–1858; Fig. 3A.

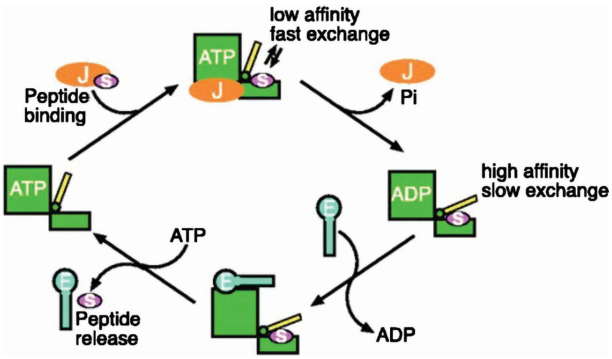

Fig. 7.2 Reaction cycle of the DnaK chaperone machine. DnaK-ATP with the open lid constitutes the active form able to accept non-native substrate polypeptide chains. These are either targeted to DnaK by DnaJ (as shown here) or bind directly to the peptide-binding domain of DnaK. The initial binding of the substrate protein is characterized by low affinity allowing fast dissociation. Binding of DnaJ to DnaK stimulates its ATPase activity resulting in the release of inorganic phosphate followed by closing of the lid, as shown in the cartoon. Now, the substrate protein is trapped and can start to refold. Later, GrpE binds to the complex, stimulates release of ADP followed by opening of the lid with concomitant dissociation of the substrate protein. After binding of ATP, a new reaction cycle can be started. F.U. Hartl, M. Hayer-Hartl **2002**, *Science* 295, 1852–1858; Fig. 3B.

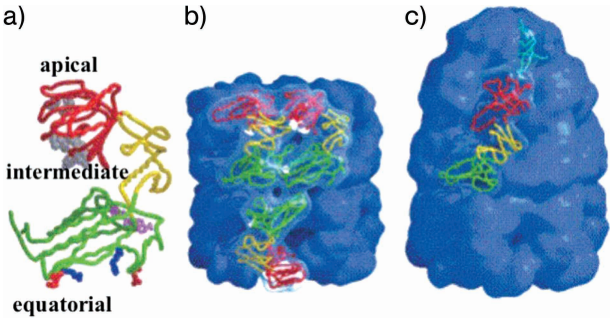

Fig. 7.3 The GroE chaperonin complex. (a) The three functional domains of the GroEL monomer. (b) Structure of two GroEL rings (14-mer) with three subunits shown at the front. (c) The GroEL-ADP-GroES complex with one GroEL and GroES subunit each. H.R. Saibil, N.A. Ranson **2002**, Trends Biochem. Sci. 27, 627–632; Fig. 2.

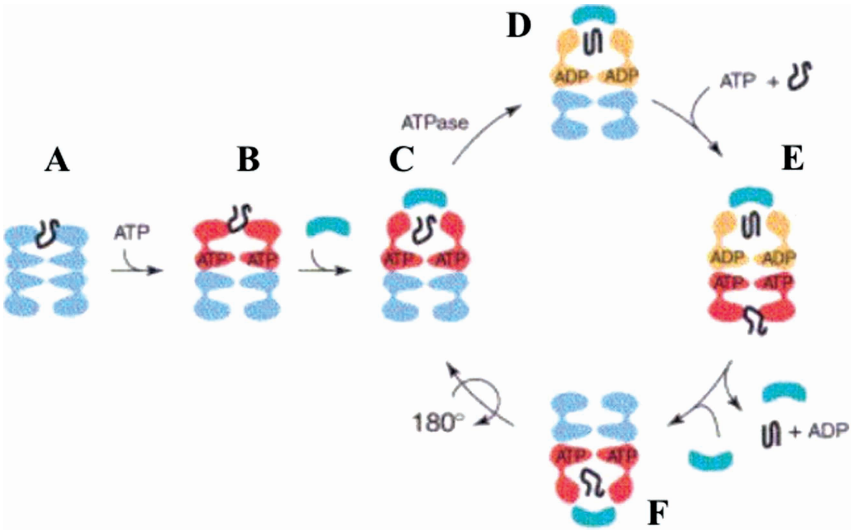

Fig. 7.4 The GroEL-GroES reaction cycle. (A) A substrate protein diffuses into the upper ring where it will bind to hydrophobic side chains exposed on the inner surface of the apical domain. (B) Next, each equatorial domain of the upper ring binds one molecule of ATP with positive cooperativity. (C) This in turn triggers binding of a GroES ring to the upper ring causing an upward movement of the apical domain, retraction of the hydrophobic side-chains and release of the substrate protein into the cavity of the upper GroEL ring where it starts to fold. (D) ATP is hydrolyzed and (E) another substrate molecule diffuses into the lower ring, followed by binding of ATP. (F) GroES diffuses away from the upper ring, followed by the substrate protein; and the substrate molecule bound to the lower ring gets encapsulated. H.R. Saibil, N.A. Ranson **2002**, Trends Biochem. Sci. 27, 627–632; Fig. 1.

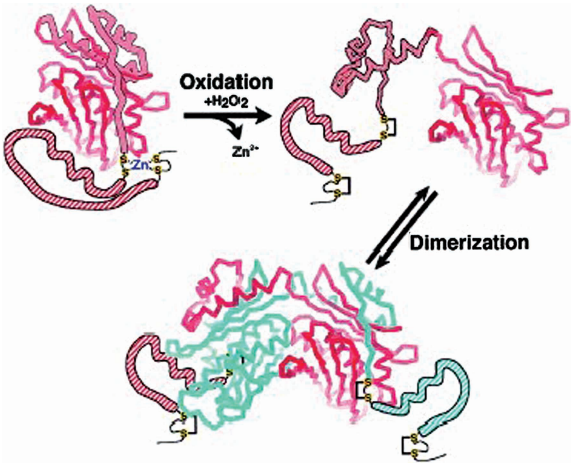

Fig. 7.8 Model of Hsp33 activation. Under reducing conditions, Hsp33 occurs in a monomeric form and four cysteine residues are involved in zinc coordination. After addition of H_2O_2, zinc is released and two intramolecular disulfide bonds are formed. Next, two oxidized Hsp33 monomers form a highly active dimer. P.C.F. Graf, U. Jacob **2002**, *Cell. Mol. Life Sci.* 59, 1624–1631; Fig. 1.

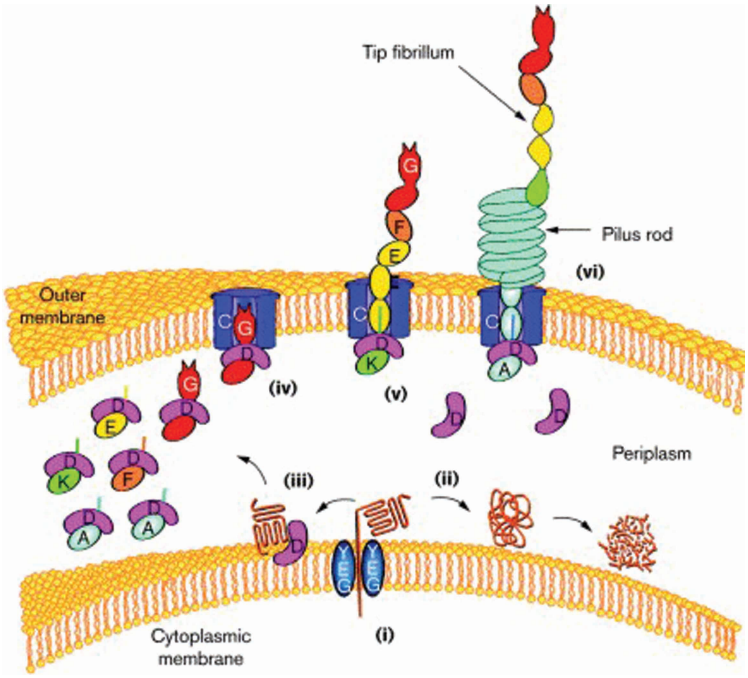

Fig. 7.9 Biogenesis of the P pilus. (i) The components of the P pilus and those involved in its biogenesis are synthesized in the cytoplasm and secreted into the periplasm using the Sec pathway. (ii) If components of the P pilus do not interact with the PapD chaperone, they fold inappropriately and are degraded by periplasmic proteases. (iii) Most P pilus components are bound by the PapD chaperone and guided to the PapC protein, an integral outer membrane protein acting as an usher (iv). (v) PapC monitors the correct assembly of the different components which form the active P pilus (vi). F.G Sauer, et al. **2000**, *Curr. Opin. Struct. Biol.* 10, 548–556; Fig. 1.

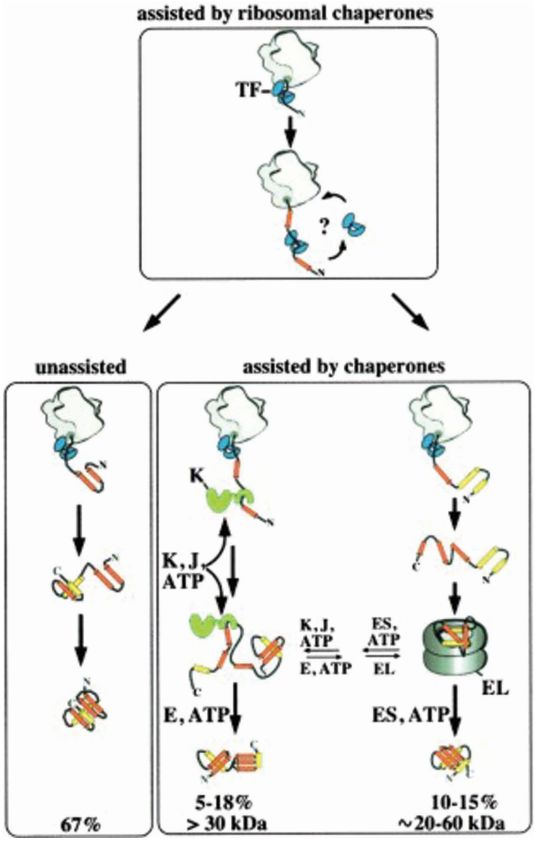

Fig. 7.13 Model for chaperone-assisted folding of nascent polypeptide chains in the cytoplasm. Nascent polypeptide chains first interact with the trigger factor (TF) loacted at the exit tunnel of the ribosome. While most proteins are able to fold in the complete absence of any folder chaperone, 5–18% are dependent on the DnaK and another 10–15% on the GroE team with overlapping specificity. B. Bukau, et al. **2000**, *Cell* 101, 119–122; Fig. 1.

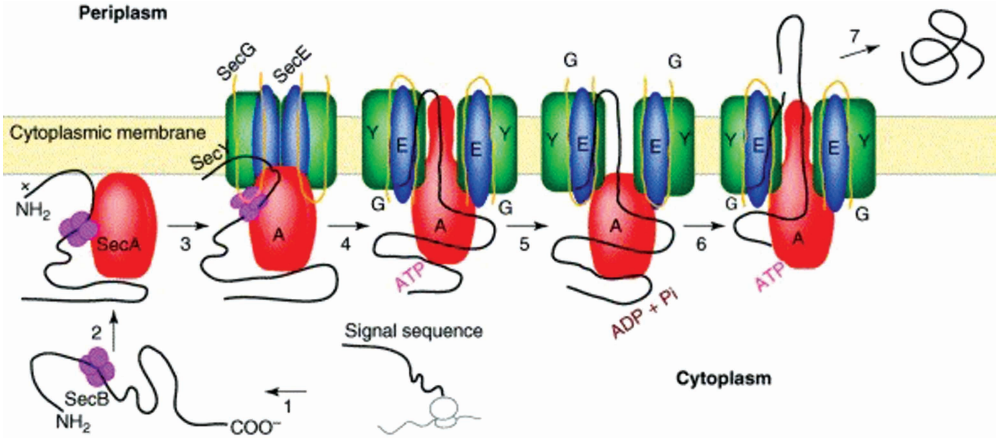

Fig. 8.5 The Sec or general translocation process. Most nascent polypeptide chains to be translocated are recognized and bound by the SecB chaperone (step 1) which target it to a SecA homodimer (step 2). Upon dissociation of SecB, SecA will bind to the inner membrane causing SecYEG to form the translocon (step 3). Next, SecA with the bound preprotein inserts into the channel (step 4) which requires bound ATP. Upon hydrolysis of ATP, SecA retracts (step 5), binds to another segment of the protein which is feeded together with SecA into the channel (step 6). During each translocation step, 20–30 amino acid residues are pushed through the translocon. H. Mori **2002**, *Trends Microbiol.* 9, 494–500; Fig. 1.

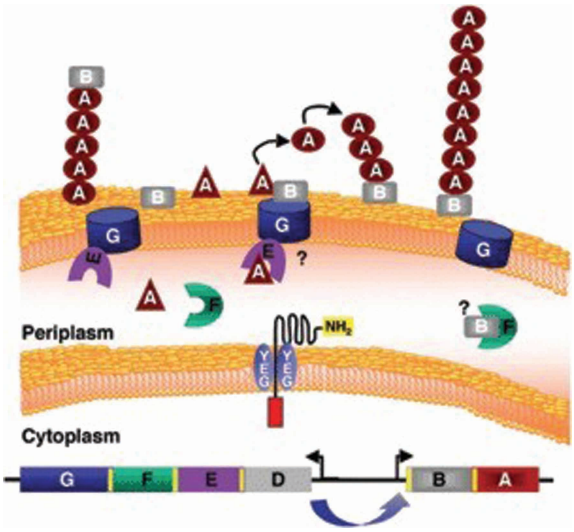

Fig. 8.10 Current model of curli fiber regulation and assembly. All curli subunits except CsgD start with a signal sequence required for translocation into the periplasm by the Sec pathway. CsgA and CsgB are the major and minor curli subunits, respectively, kept in a polymerization-competent form by the CsgF chaperone. CsgG might form a pore in the outer membrane through which CsgB and CsgA escape where CsgB acts as a nucleator. CsgE could transfer CsgA and CsgB to CsgG as indicated. M.R. Chapman, et al. **2003**, *ASM News* 69, 121–126; Fig. 2.

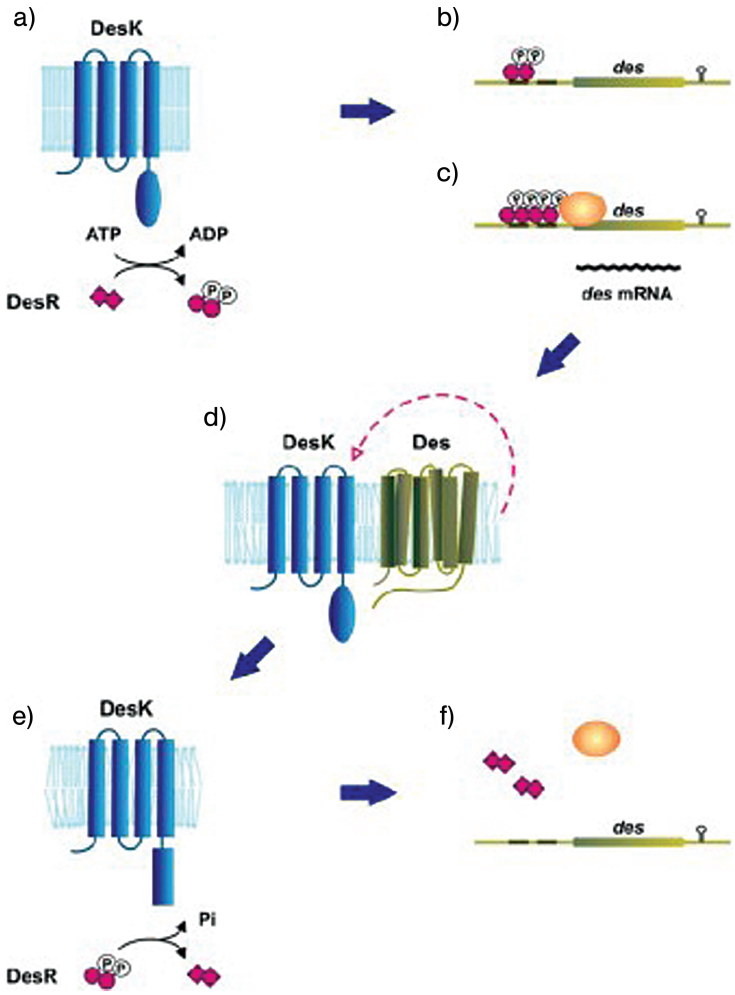

Fig. 9.6 Model of the Des pathway. (a) If cells experience a temperature decrease to 25 °C, this is sensed by DesK and leads to the activation of its kinase. DesK undergoes autophosphorylation and then transfers the phosphoryl group to the response regulator DesR. (b, c) The active DesR~P binds to DNA sites immediately upstream of the *des* promoter to initiate transcription by interaction with the RNA polymerase. (d) The desaturase inserts into the cytoplasmic membrane and introduces double bonds into acyl chains of the phospholipids which converts DesK from a kinase to a phosphatase. (e) Dephosphorylation of DesR~P results in turn-off of *des* transcription. M.C. Mansilla, et al. **2005**, *Arch. Microbiol.* 183, 229; Fig. 2.