Prof. Giorgio Sartor

Motori molecolari

V – Flagello batterico

M09 - Versione 1.8 – 03/10/2018

I FLAGELLI NEI BATTERI

Un diverso movimento
Un diverso carburante

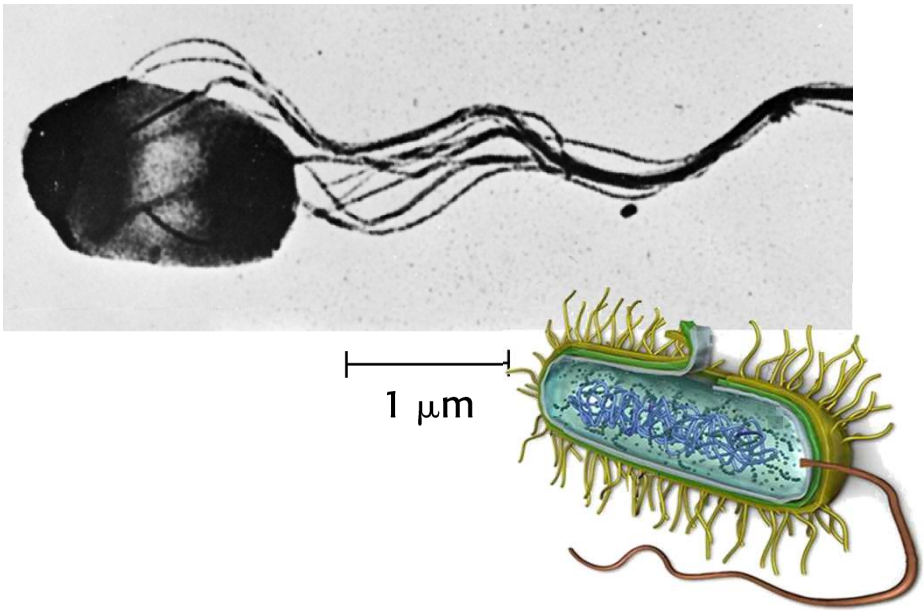
Schienenzeppelin (1929-1939)

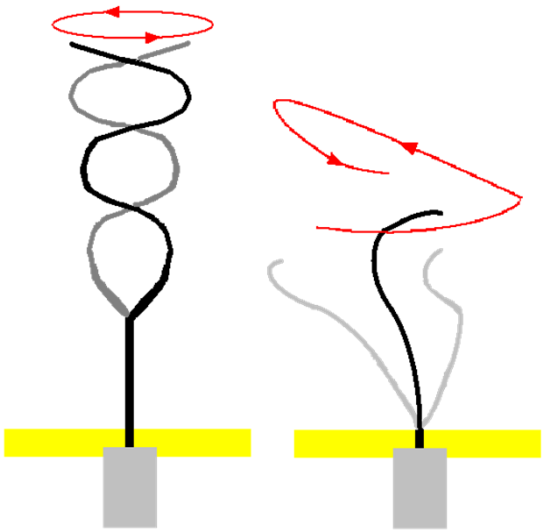
Per muoversi in un liquido

- Alcuni batteri possiedo un sistema estremamente sofisticato per muoversi in acqua:

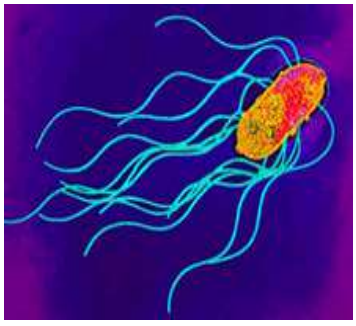
I flagelli

- Non c'è nulla di simile in cellule più complesse.
- Il flagello agisce come un'elica che ruota.



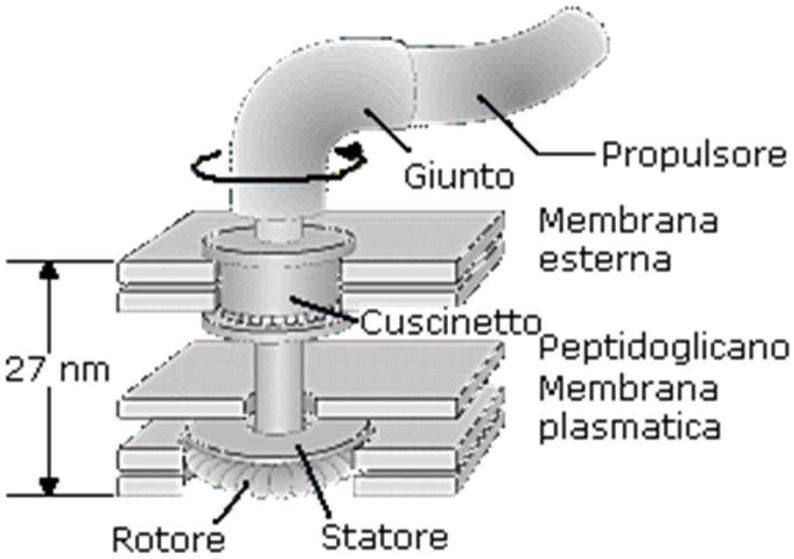


I flagelli

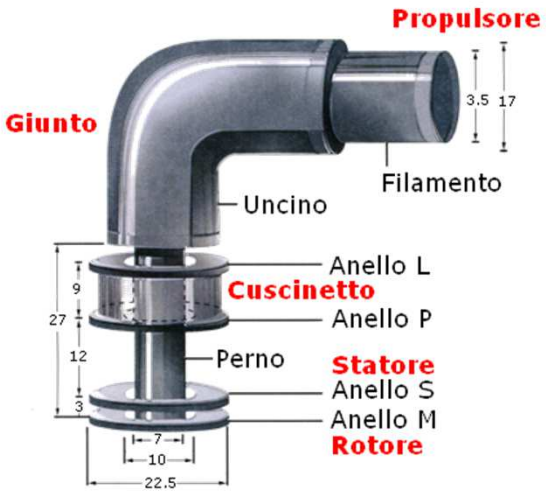


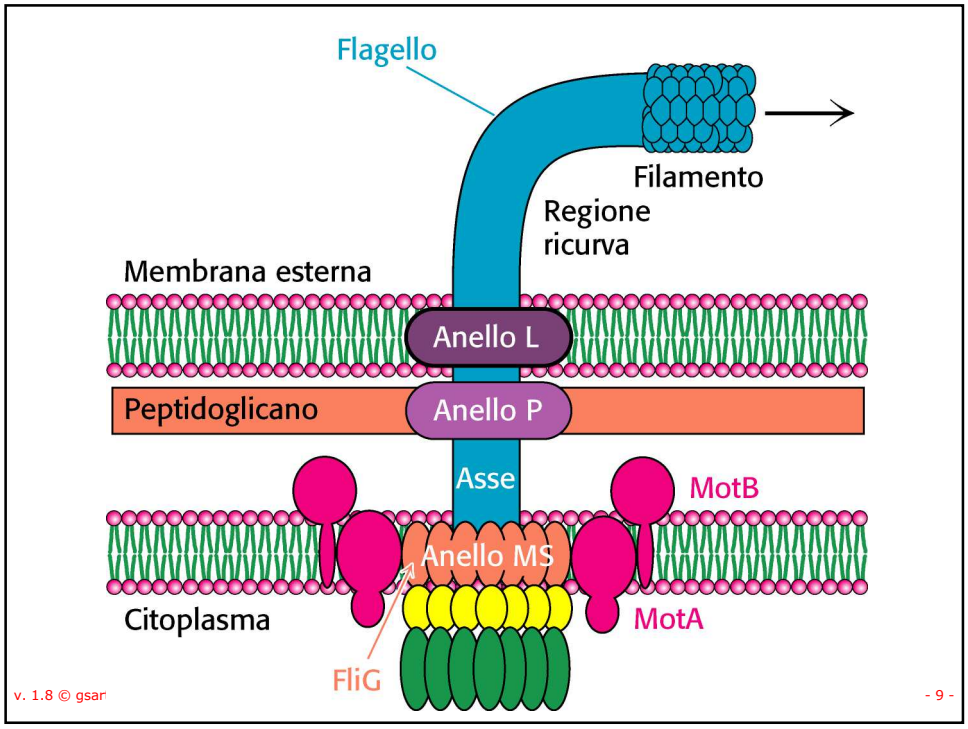
- Usati per il movimento di batteri in ambiente acquatico
- Permettono il movimento in risposta ad uno stimolo ambientale:
  - Chimico (chemotassi)
  - Luminoso (fototassi)
  - Calore (termotassi)
  - Campo magnetico (magnetotassi)
  - … (…tassi)

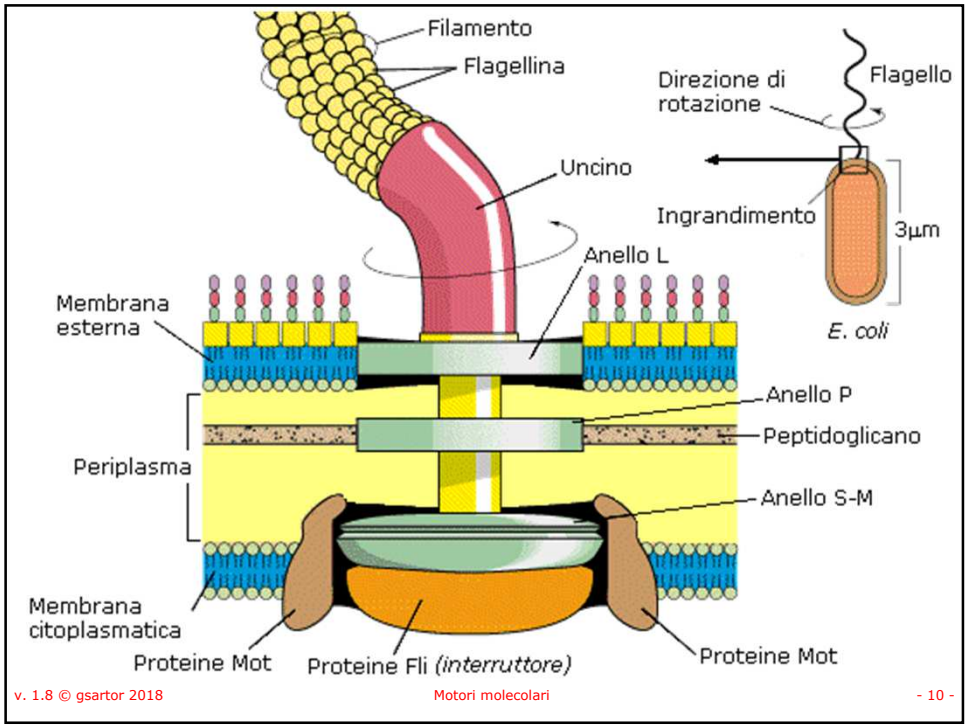
Modello concettuale



Modello concettuale e realtà




Flagello
Filamento
Regione ricurva
Membrana esterna
Anello L
Peptidoglicano
Anello P
Asse
MotB
Anello MS
Citoplasma
MotA
FliG

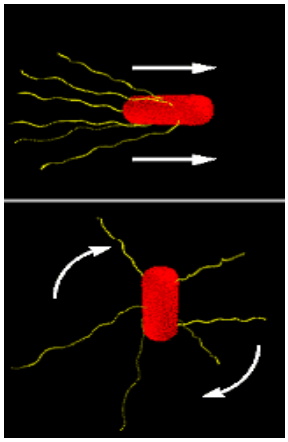

Filamento
Flagellina
Direzione di rotazione
Flagello
Uncino
Ingrandimento
3µm
Anello L
E. coli
Membrana esterna
Anello P
Peptidoglicano
Periplasma
Anello S-M
Membrana citoplasmatica
Proteine Mot
Proteine Fli (interruttore)
Proteine Mot

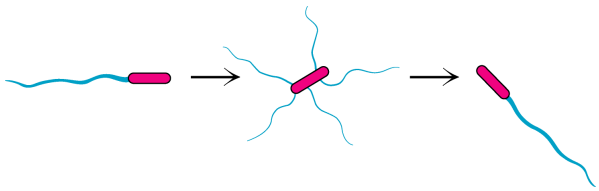
Flagello batterico

- È il motore più piccolo e più complesso
- Ha un diametro di 30 nm;
- Ruota a 270 RPS (16200 RPM);
- Efficienza energetica vicina al 100%;
- Fa ruotare il filamento attraverso una serie di anelli;
- Può ruotare in senso orario o antiorario
- Una proteina(e) alla base del motore gestisce il senso di rotazione
- Prototipo per la nanotecnologia:
- http://www.nanonet.go.jp/english/mailmag/2004/files/011a.wmv

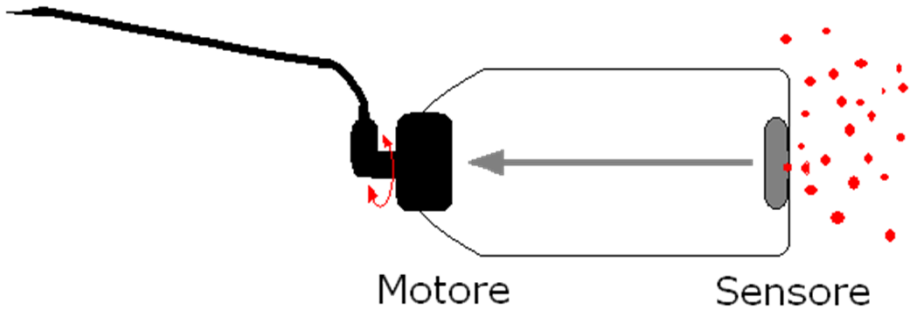
Come funziona?

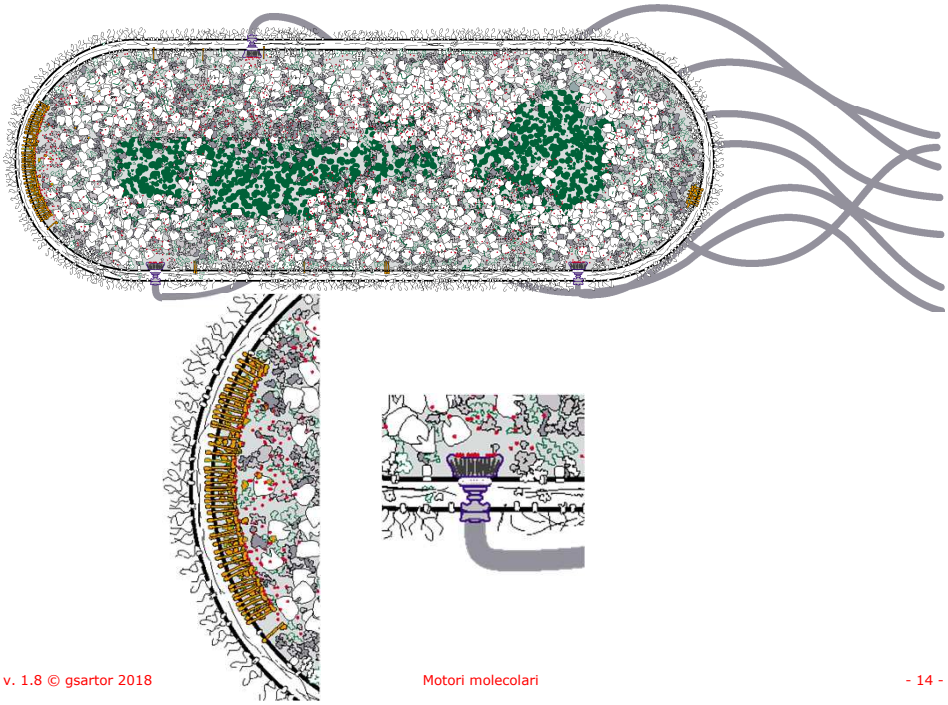
- La rotazione antioraria del flagello genera un movimento rettilineo senza cambio di di direzione;
- Una rotazione lenta in senso orario genera un rotolamento del batterio e cambia la direzione del movimento.



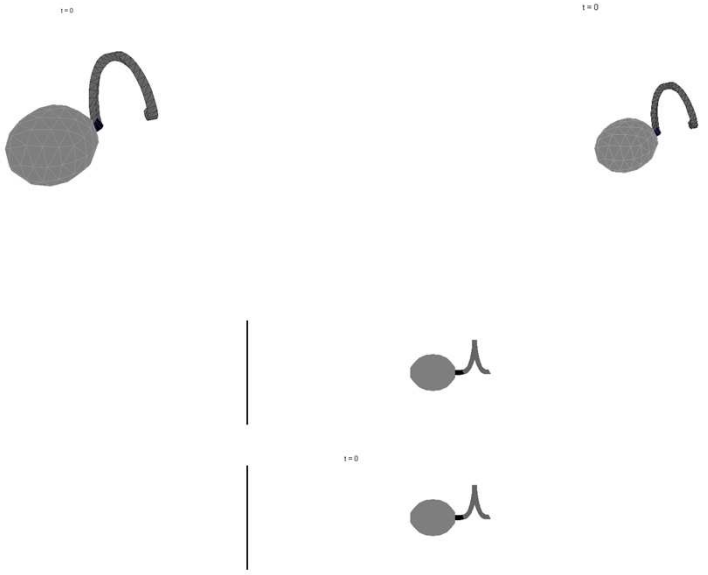


Sentendo l'ambiente

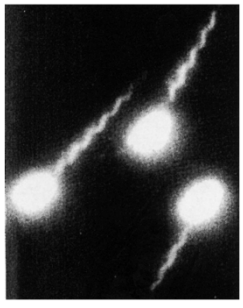




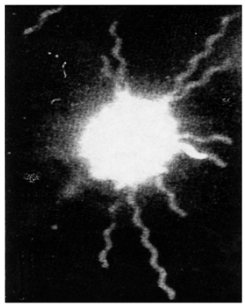
Il movimento simulato

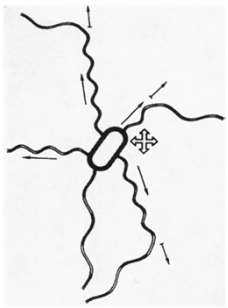


Movimento rettilineo



Rotolamento

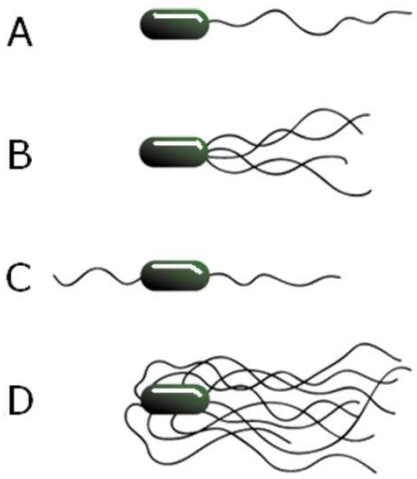


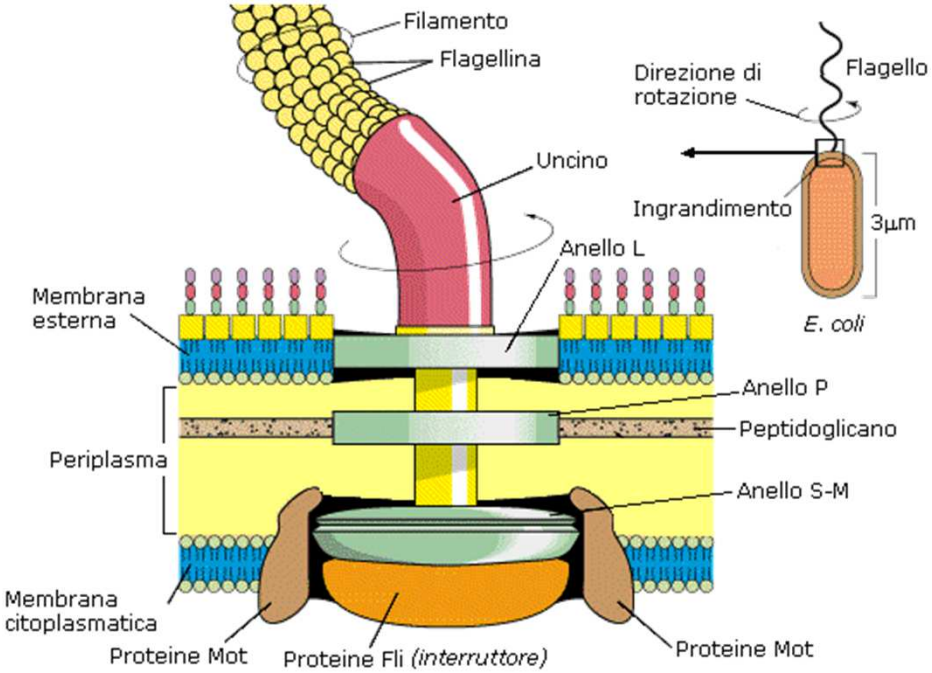


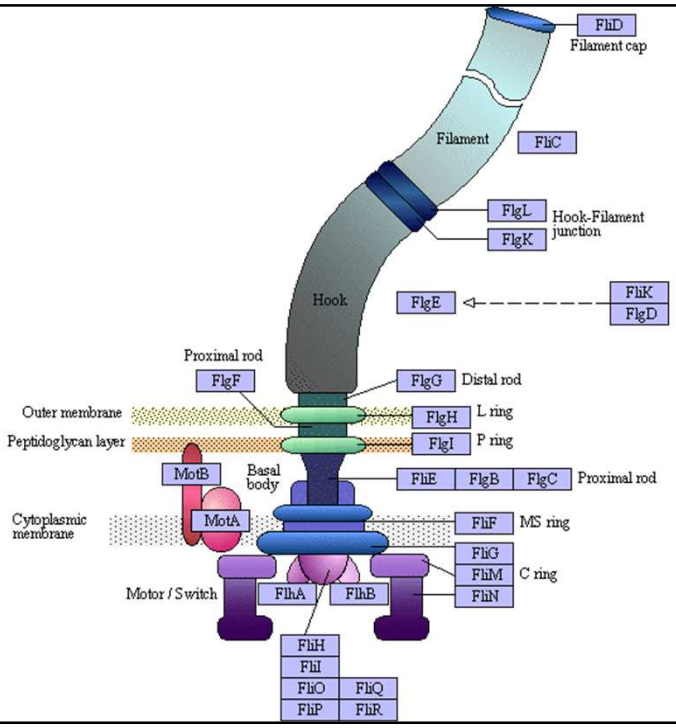
http://www.rowland.org/labs/bacteria/showmovie.php?mov=fluo_fil_leave

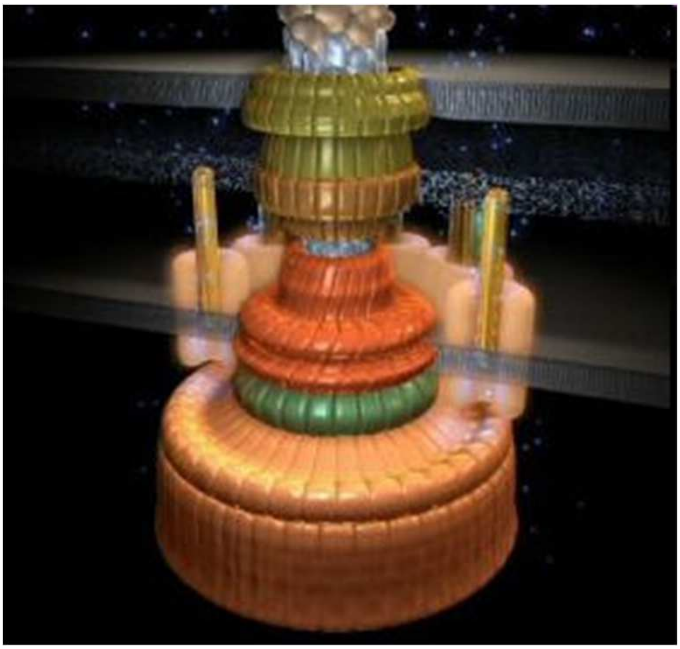
Varietà di flagelli

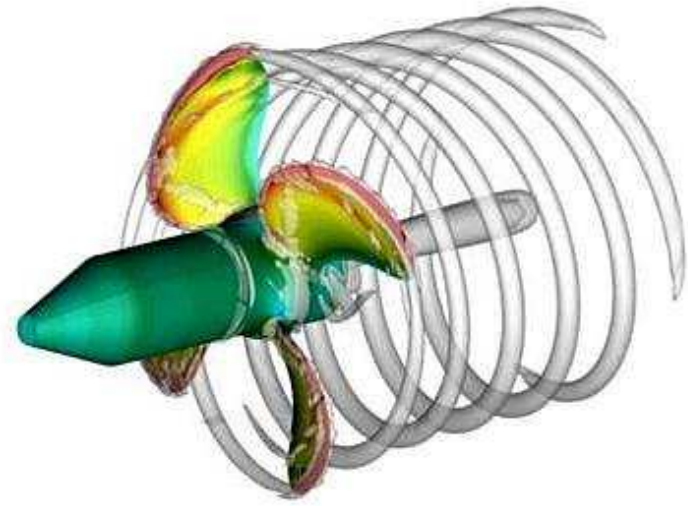
A. Un singolo flagello ad una estremità della cellula batterica (*monotrichous*);
B. Vari flagelli ad una o all'altra estremità della cellula batterica (*lophotrichous*);
C. Un singolo flagello ad ogni estremità della cellula (*amphitrichous*);
D. Vari flagelli sono distribuiti sull'intera superficie della cellula batterica (*peritrichous*).



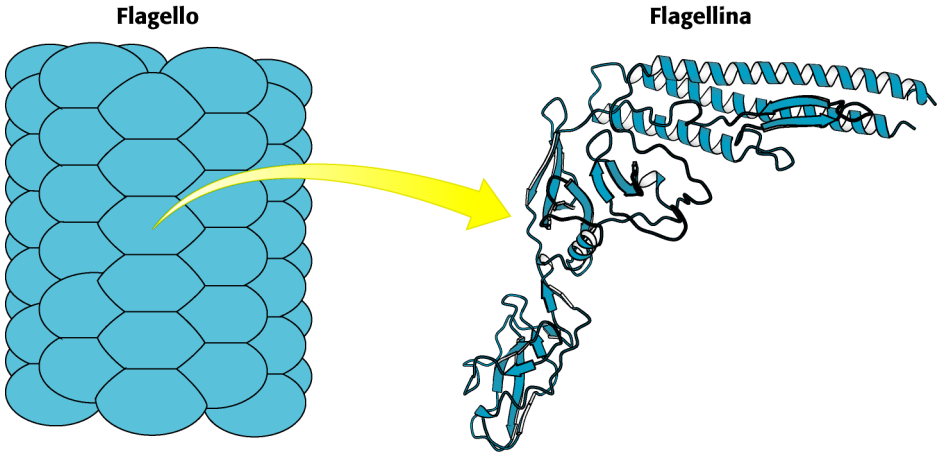



FliD
Filament cap
Filament
FliC
FlgL
FlgK
Hook-Filament junction
Hook
FlgE
FliK
FlgD
Proximal rod
FlgF
FlgG
Distal rod
Outer membrane
FlgH
L ring
Peptidoglycan layer
FlgI
P ring
MotB
Basal body
FliE
FlgB
FlgC
Proximal rod
Cytoplasmic membrane
MotA
FliF
MS ring
FliG
FliM
FliN
C ring
Motor / Switch
FlhA
FlhB
FliH
FliI
FliO
FliQ
FliP
FliR



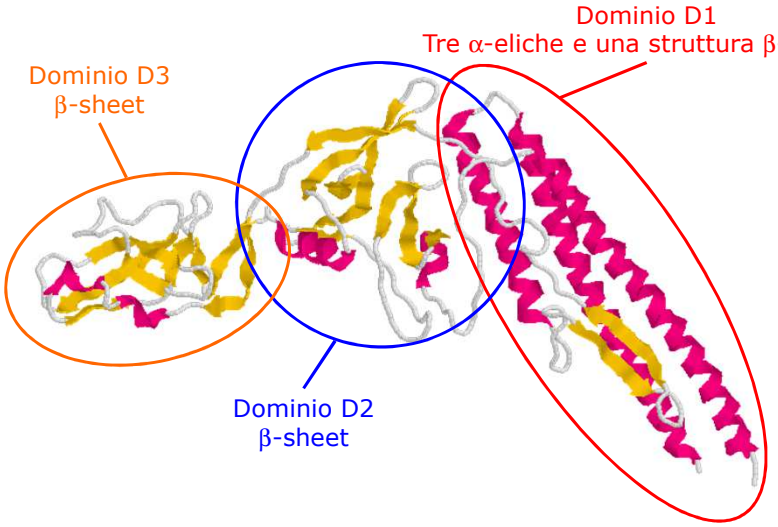


Struttura del flagello



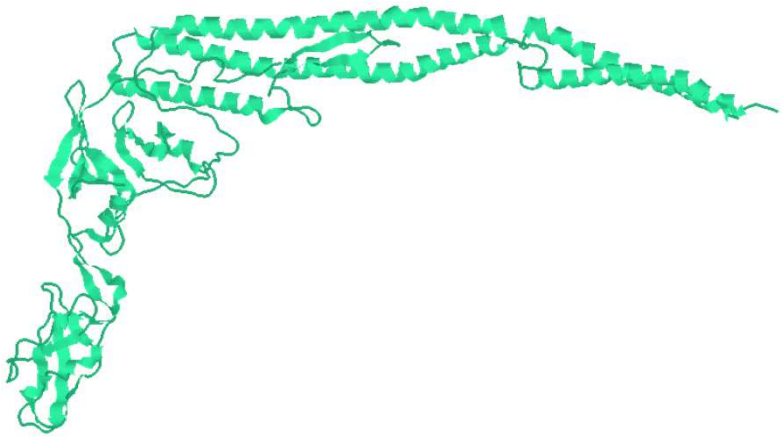
20000-30000 per flagello

Flagellina



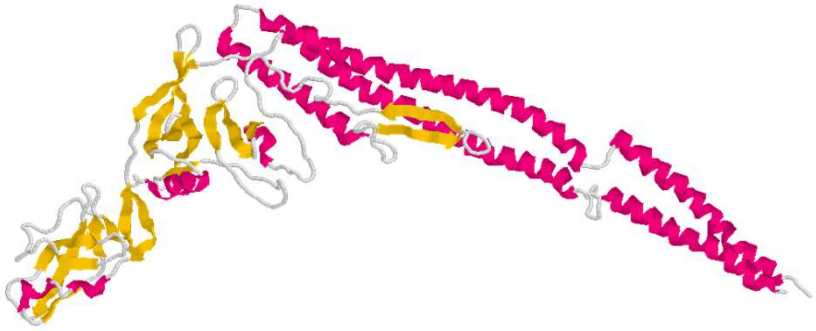
1io1

Flagellina



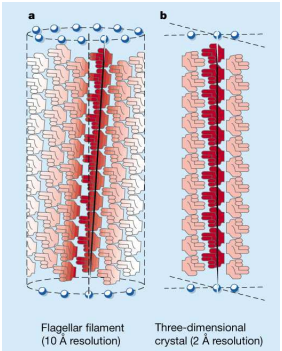
3a5x

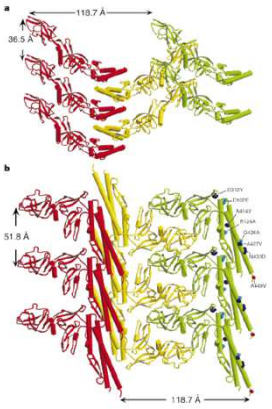
Flagellina



1ucu

Filamento di flagellina





Structure of the bacterial flagellar protofilament and implications for a switch for supercoiling

Fadel A. Samatey*, Katsumi Imada*, Shigehiro Nagashima*, Ferenc Vonderviszt†, Takashi Kumasaka‡, Masaki Yamamoto‡ & Keiichi Namba*§

NATURE | VOL 410 | 15 MARCH 2001

Figure 3 Crystal packing of F41 in two orthogonal views. **a**, The *b*–*c* plane viewed down the *a* axis. **b**, The *a*–*c* plane viewed down the *b* axis. Each array of the F41 molecules along the *a* axis, which has been identified as the protofilament of the flagellar filament, is coloured red, yellow and green. The repeat distances are labelled. Antiparallel packing of the protofilaments by the $P2_1$ symmetry of the crystal is clearly shown. Mutation sites that affect the supercoiling are also labelled with side-chain atoms shown in CPK representation. Mutations are coloured blue for L-type, red for R-type, and black for curly. Prepared with MOLSCRIPT[44] and RASTER3D[45].

Filamento di flagellina

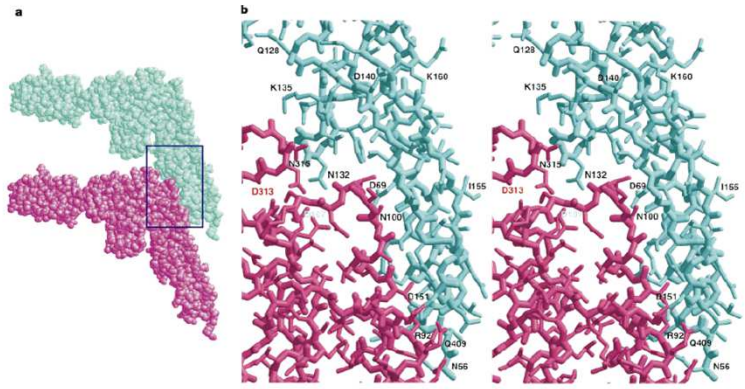


Figure 5 Axial interactions in the protofilament. **a**, Space-filling representation showing the axial packing of two subunits. Prepared with RASMOL[46]. **b**, Stereo close-up view of the boxed region in **a**. The two subunits shown are coloured cyan (upper) and dark pink (lower). Some of the residues are labelled to guide identification. Prepared with MOLSCRIPT[44] and RASTER3D[45].

Structure of the bacterial flagellar protofilament and implications for a switch for supercoiling

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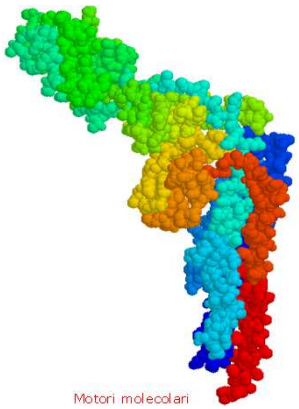
Assemblaggio della flagellina

Movimento molecolare

1 http://molvis.sdsc.edu/flagella/1io1_1ucu_morph_bb.htm

2 http://molvis.sdsc.edu/flagella/1io1_1ucu_morph_sf.htm



1io1 – 1ucu

Assemblaggio della flagellina

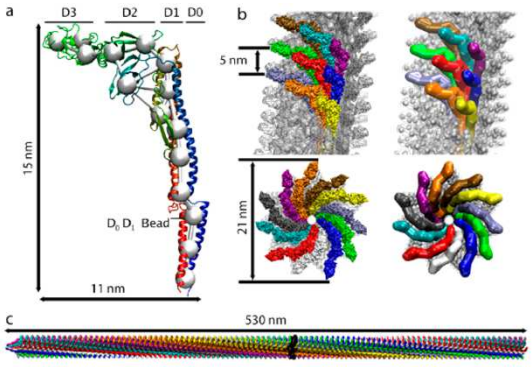


FIGURE 1 Coarse-graining scheme for the flagellar filament. In panel *a*, a flagellin monomer is shown in both all-atom (*cartoon*) and CG (*white beads*) representations. The CG model consists of 15 beads placed according to the mass distribution in the protein; two beads are bonded if the distance between them is <25 Å. The different domains of the flagellin monomer are noted (*top*). In panel *b*, the flagellar filament viewed from the side and from the top is shown in all-atom (*left*) and CG (*right*) representations. A few monomers are highlighted in various colors, to demonstrate the arrangement of monomers comprising the filament. The longest simulated filament segment (1100 monomers) is shown in CG representation in panel *c*. The filament is built of monomers arranged in a helical fashion, 11 proteins per helix turn (single helix turn is highlighted in *black*). The 11 protofilaments, all in the R state, are drawn in differing colors.

Biophysical Journal Volume 91 December 2006 4589–4597

Coarse-Grained Molecular Dynamics Simulations of a Rotating Bacterial Flagellum

Anton Arkhipov,*† Peter L. Freddolino,†‡ Katsumi Imada,§¶ Keiichi Namba,§¶ and Klaus Schulten*‡

Assemblaggio della flagellina

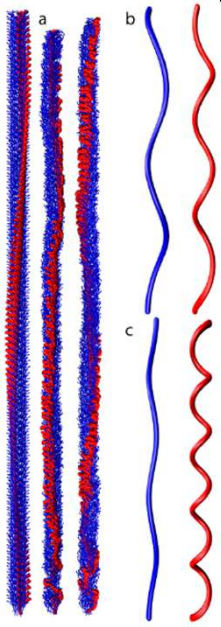
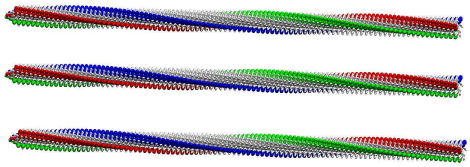


FIGURE 3 Coiling and supercoiling of the 1100-monomer flagellar filament segment in our simulations. (*a*) Full view of the starting structure (*left*), conformation of the segment after 25 μs in simulation F1100swim (*center*), and after 25 μs in simulation F1100tumble. In each case a single protofilament is highlighted in red to illustrate the protofilament coiling. (*b*) Schematic view of the supercoiling observed after 25 μs in simulations F1100swim (*blue*) and F1100tumble (*red*), with an exaggerated supercoil diameter to better illustrate the nature of the helix. Helical parameters were determined as discussed in the text. (*c*) Schematic view (as in *b*) of supercoil models, constructed using the bistable protofilament model with protofilament heights and angles obtained from our simulations.



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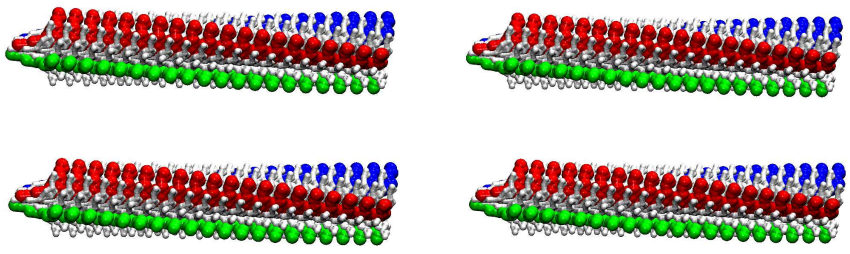
Movimento del propulsore



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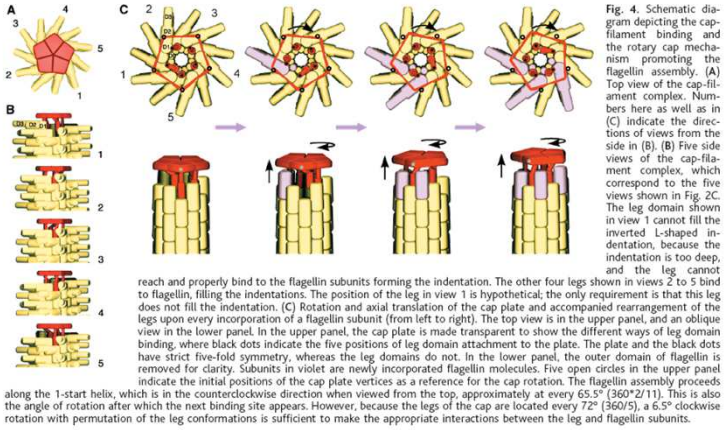


Fig. 4. Schematic diagram depicting the cap-filament binding and the rotary cap mechanism promoting the flagellin assembly. (A) Top view of the cap-filament complex. Numbers here as well as in (C) indicate the directions of views from the side in (B). (B) Five side views of the cap-filament complex, which correspond to the five views shown in Fig. 2C. The leg domain shown in view 1 cannot fill the inverted L-shaped indentation, because the indentation is too deep, and the leg cannot reach and properly bind to the flagellin subunits forming the indentation. The other four legs shown in views 2 to 5 bind to flagellin, filling the indentations. The position of the leg in view 1 is hypothetical; the only requirement is that this leg does not fill the indentation. (C) Rotation and axial translation of the cap plate and accompanied rearrangement of the legs upon every incorporation of a flagellin subunit (from left to right). The top view is in the upper panel, and an oblique view in the lower panel. In the upper panel, the cap plate is made transparent to show the different ways of leg domain binding, where black dots indicate the five positions of leg domain attachment to the plate. The plate and the black dots have strict five-fold symmetry, whereas the leg domains do not. In the lower panel, the outer domain of flagellin is removed for clarity. Subunits in violet are newly incorporated flagellin molecules. Five open circles in the upper panel indicate the initial positions of the cap plate vertices as a reference for the cap rotation. The flagellin assembly proceeds along the 1-start helix, which is in the counterclockwise direction when viewed from the top, approximately at every 65.5° (360°2/11). This is also the angle of rotation after which the next binding site appears. However, because the legs of the cap are located every 72° (360/5), a 6.5° clockwise rotation with permutation of the leg conformations is sufficient to make the appropriate interactions between the leg and flagellin subunits.

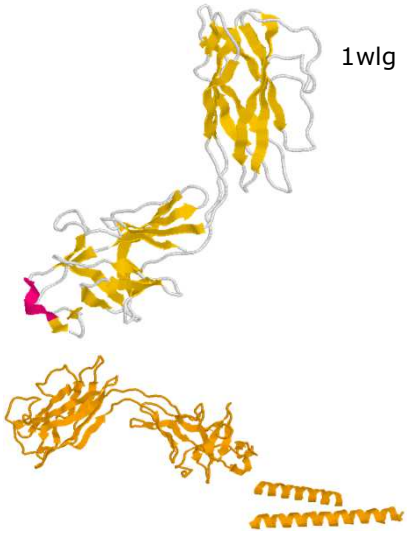
The Bacterial Flagellar Cap as the Rotary Promoter of Flagellin Self-Assembly

Koji Yonekura,[1*] Saori Maki,[1*] David Gene Morgan,[2] David J. DeRosier,[3] Ferenc Vonderviszt,[4] Katsumi Imada,[1] Keiichi Namba[1,5†]

SCIENCE VOL 290 15 DECEMBER 2000

Struttura dell'uncino

- L'uncino del flagello è una corta struttura tubulare che connette il motore rotativo con il flagello;
- La flessibilità dell'uncino gli permette di funzionare come un giunto.

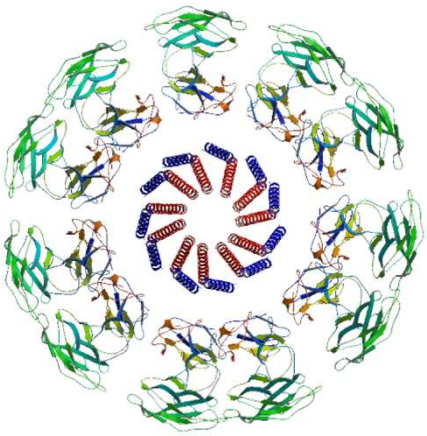


1wlg

3a69

Struttura dell'uncino

- L'uncino del flagello è una corta struttura tubulare che connette il motore rotativo con il flagello;
- La flessibilità dell'uncino gli permette di funzionare come un giunto.



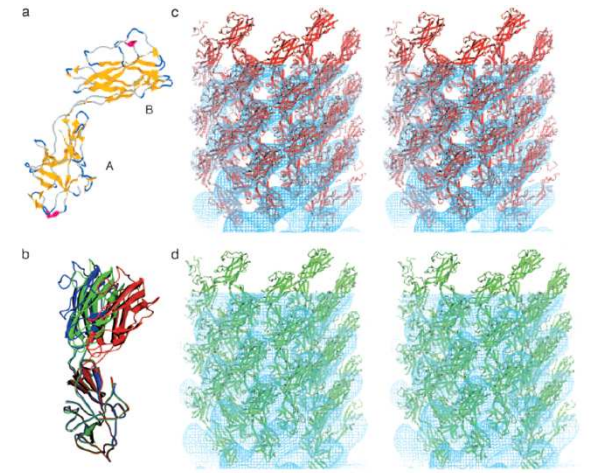


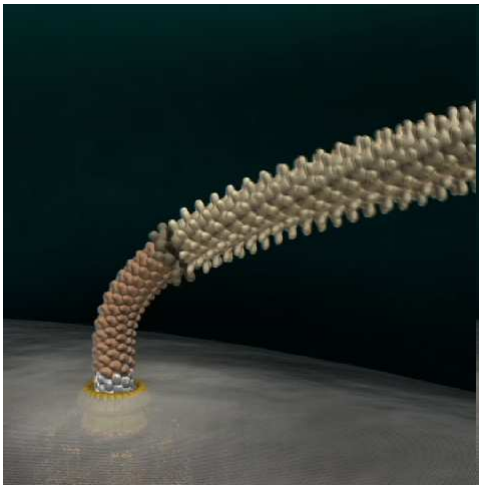
Fig. 3. The atomic model of the outer two domains of the hook. (*a*) The structure of a major fragment of the hook subunit as determined by x-ray crystallography (28). β-Sheet is shown in yellow and α-helical segments in red. The lower domain (A) contains both N- and C-terminal regions, has the more evolutionarily conserved amino acid sequence, and corresponds to domain D1. The upper domain (B) corresponds to D2. (*b*) The change in domain arrangement involved in the docking and refinement. Three models are superimposed by fitting the D1 domains to one another. The figure shows the difference in the angle between domains D1 and D2 after refinement. The crystal structure model is shown in blue; the model refined against the *C. crescentus* map, green; the model refined against the *S. typhimurium* map, red. (*c*) A stereo pair of the atomic structure docked into the *S. typhimurium* map. Some of the subunits are shown outside the map. (*d*) A stereo pair showing the atomic structure docked into the *C. crescentus* map.

A partial atomic structure for the flagellar hook of *Salmonella typhimurium*

Tanvir R. Shaikh*†, Dennis R. Thomas*, James Z. Chen*, Fadel A. Samatey‡§¶, Hideyuki Matsunami‡§¶, Katsumi Imada‡§¶, Keiichi Namba‡§¶, and David J. DeRosier*||

PNAS | January 25, 2005 | vol. 102 | no. 4 | 1023–1028

Uncino



http://www.youtube.com/watch?v=Ey7Emmddf7Y&feature=related

Rotore

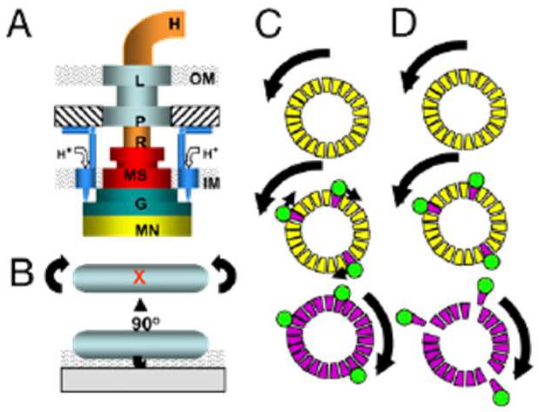


Fig. 1. (*A*) Schematic view of the *E. coli* flagellar motor. Rotor elements are as follows: MN (FliMN), G (FliG), MS (MS ring), R (rod), and H (hook). Stationary elements are P (P ring), L (L ring), and M ($MotA_4MotB_2$) complex. Mot complexes attach to the cell wall (hatched), conduct H^+ through the membrane, and interact with FliG. IM and OM denote the inner and outer membranes. (*B*) Cartoon of a tethered cell. A sheared flagellar filament is affixed by using antifilament antibody. The cell body counter rotates to the direction of motor rotation. The red X marks the point at which the tethering flagellum is located. The wavy pattern indicates the evanescent wave generated by TIRF. (*C*) The domino model for cooperative switching within the motor. The motor at the top is spinning CCW. CheY-P binding to FliM (trapezoids) initiates a wave of conformational change, shown as CW-facing arrows, to place the C ring in the CW conformation. (*D*) FliM destabilization model for cooperative switching. CheY-P (green circle) binding to FliM destabilizes the FliM ring, which allows the motor to "relax" into the CW conformation. Both models are speculative, and the scenario shown in *D* exists only in the imagination of the author.

Dynamic motors for bacterial flagella

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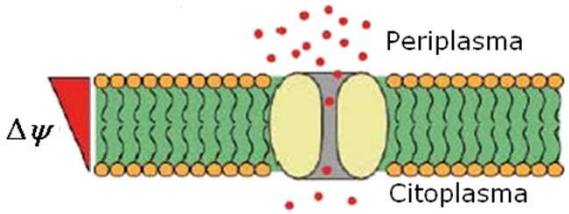
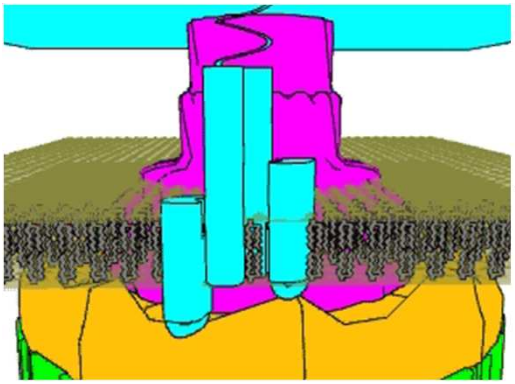
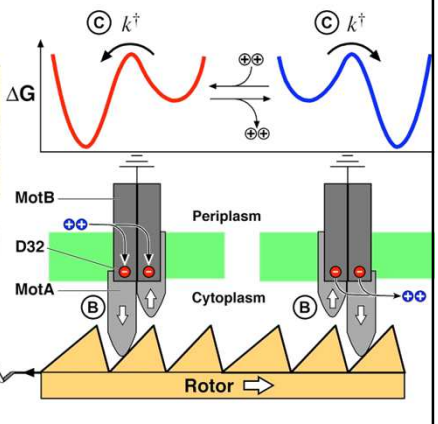
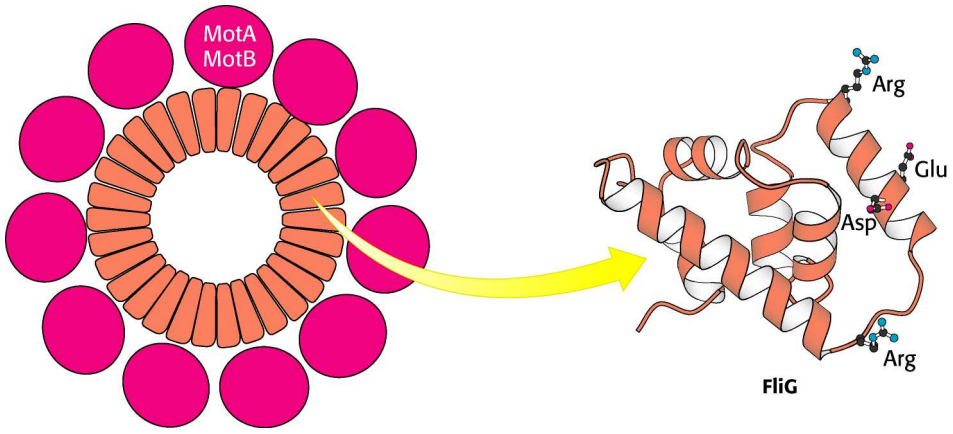
Il carburante

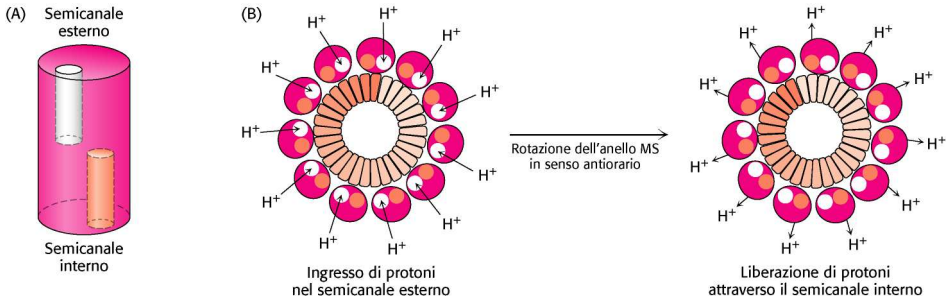
$$pmf = \underbrace{\Delta\psi}_{\text{Potenziale di membrana}} + \underbrace{\frac{k_B T}{e}\ln(C_p/C_c)}_{\text{Grediente di concentrazione ionica}}$$



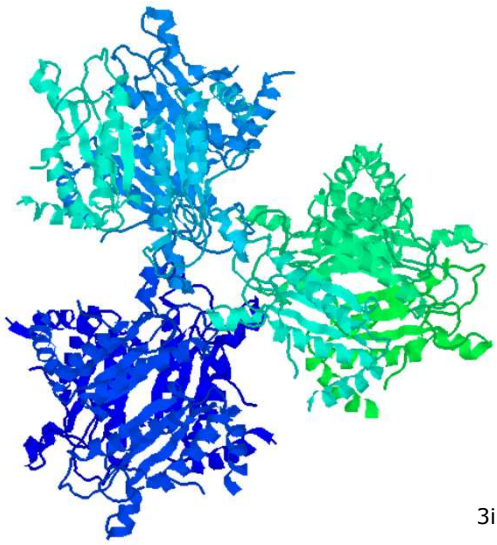



ΔG
MotB
Periplasm
D32
MotA
Cytoplasm
Load
Elastic coupling
Rotor


MotA
MotB
Arg
Glu
Asp
Arg
FliG



Dominio C-terminale MotB (*Helicobacter pylori*)

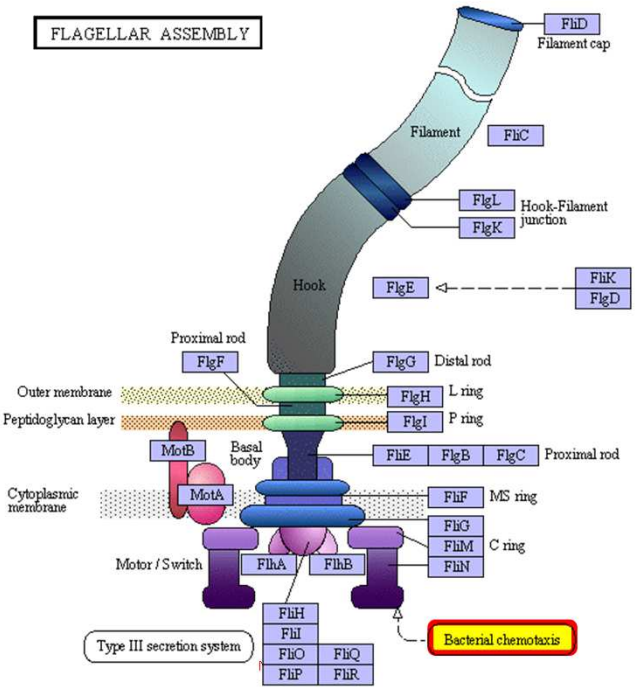


3imp

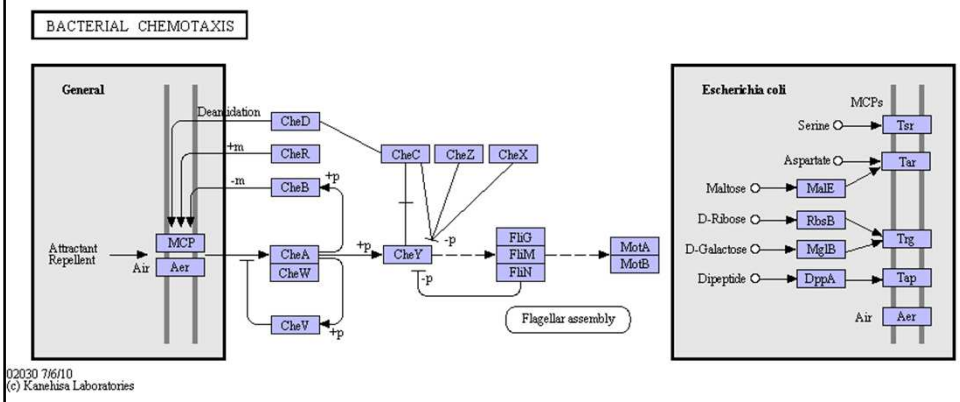
Scheda tecnica del motore

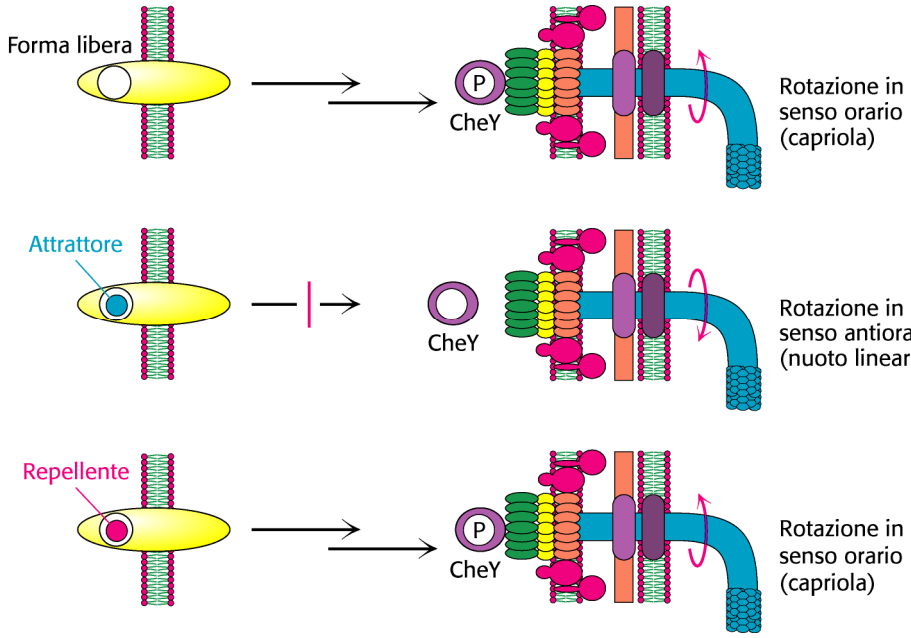
Forza propulsiva	Gradiente elettrochimico (H^+ o Na^+)
Numero di protoni per giro	~ 1000
Energia per protone	~ 2.5 x 10^{-20} J
Massima velocità di rotazione	300 Hz (H^+) 1700 Hz (Na^+)
Momento torcente in stallo	~ 4 x 10^{-18} Nm (4 nN nm)
Potenza massima	~ 10^{-15} W
Efficienza	50-100% (stallo) ~ 5% (cellula che nuota)
Numero di passi per giro	~ 50 per rotazione

http://www.nanonet.go.jp/english/mailmag/2004/files/011a.wmv (35')


FLAGELLAR ASSEMBLY
FliD
Filament cap
Filament
FliC
FlgL
FlgK
Hook-Filament junction
Hook
FlgE
FliK
FlgD
Proximal rod
FlgF
FlgG
Distal rod
Outer membrane
FlgH
L ring
Peptidoglycan layer
FlgI
P ring
MotB
Basal body
FliE
FlgB
FlgC
Proximal rod
Cytoplasmic membrane
MotA
FliF
MS ring
FliG
FliM
FliN
C ring
Motor / Switch
FlhA
FlhB
FliH
FliI
FliO
FliQ
FliP
FliR
Type III secretion system
Bacterial chemotaxis

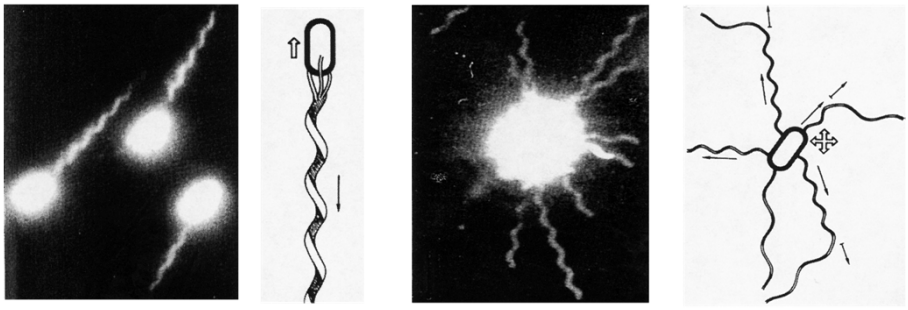
Chemotassi


BACTERIAL CHEMOTAXIS
General
Deamidation
CheD
+m
CheR
-m
CheB
+p
CheC
CheZ
CheX
Attractant
Repellent
Air
MCP
Aer
CheA
CheW
CheV
+p
+p
CheY
-p
-p
FliG
FliM
FliN
MotA
MotB
Flagellar assembly
Escherichia coli
MCPs
Serine
Tsr
Aspartate
Tar
Maltose
MalE
D-Ribose
RbsB
D-Galactose
MglB
Trg
Dipeptide
DppA
Tap
Air
Aer
02030 7/6/10
(c) Kanehisa Laboratories



Movimento rettilineo

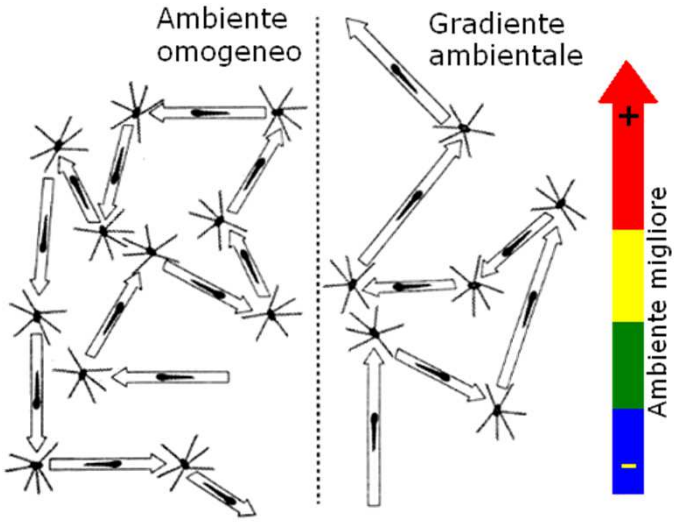
Rotolamento



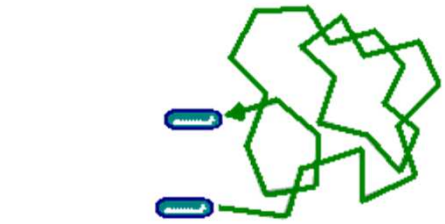
http://www.youtube.com/watch?v=Ey7Emmddf7Y&feature=related

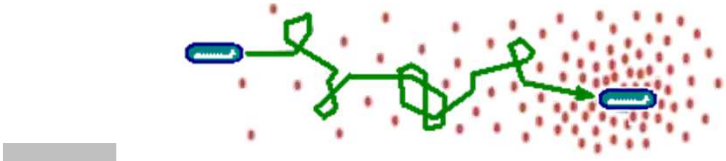
http://www.rowland.org/labs/bacteria/showmovie.php?mov=fluo_fil_leave

Frequenza di rotazione

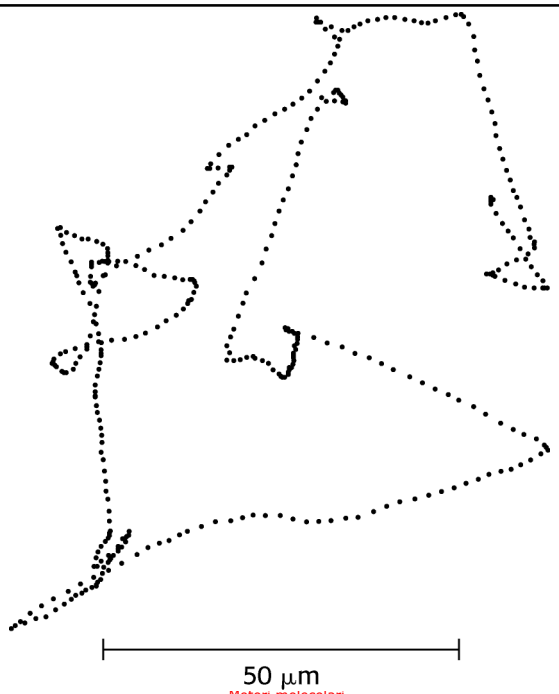


Percorso





http://virtuallaboratory.colorado.edu/Biofundamentals/labs/Adaptation/section_02.html



Stimolo - Risposta

Stimolo ambientale

↓

Recettore del segnale

↓

Sensore (Kinasi)

↓

Fosforilazione

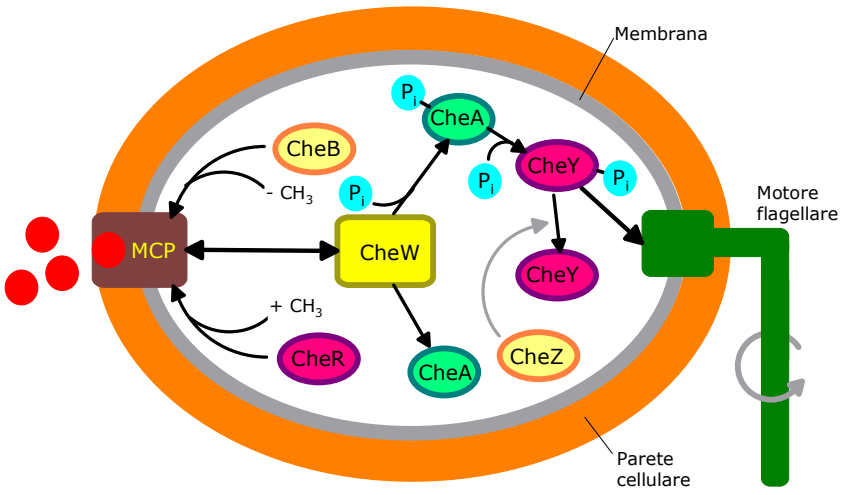
↓

Regolatore della risposta (RR)

↓

Risposta

Meccanismo della chemotassi

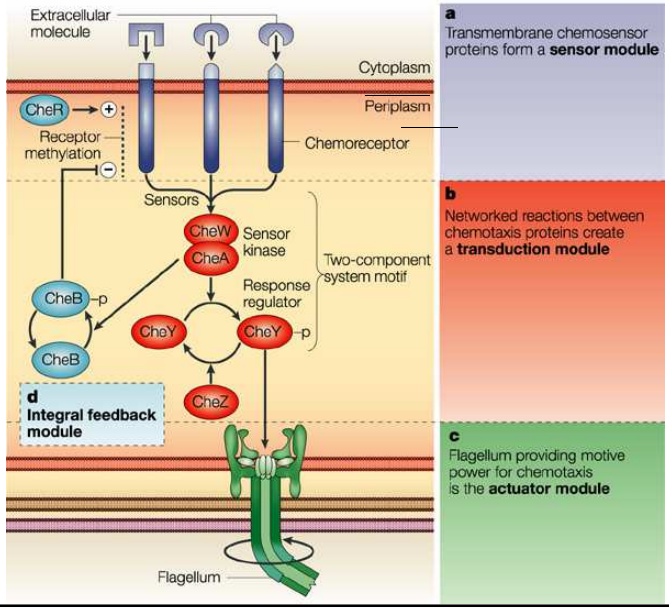


MCP: methyl-accepting chemotaxis proteins

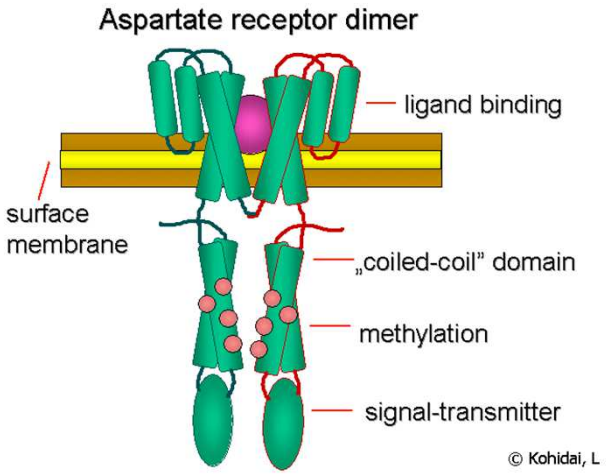
Via del segnale chemotassico in *E. coli* e *Salmonella*

- Proteine
  - MCP: Recettore
  - CheA: Histidine protein-kinase (HPK)
  - CheY: Regolatore di risposta ($CheY^P$)
  - CheZ: Fosfatasi
  - CheW: facilita l'interazione MCP e HPK
  - CheB: metilesterasi per MCP
  - CheR: metiltransferasi per MCP

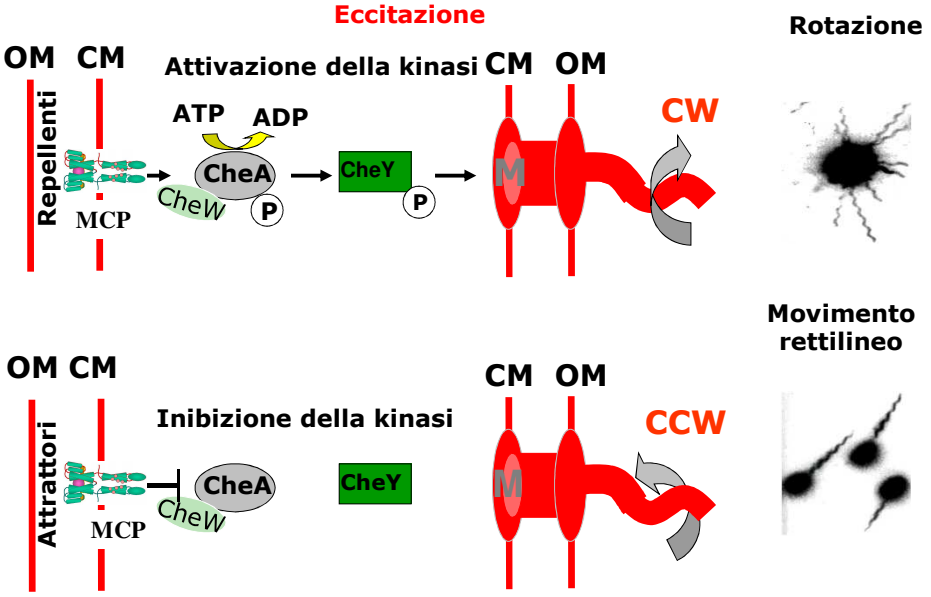
Via del segnale chemotassico in *E. coli* e *Salmonella*



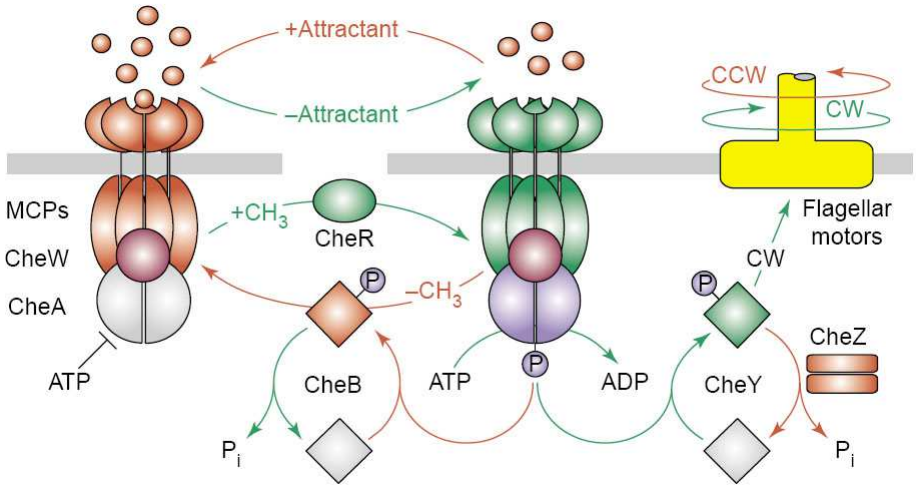
MCP (Methyl accepting Chemotaxis Proteins)



Chemotassi a due componenti

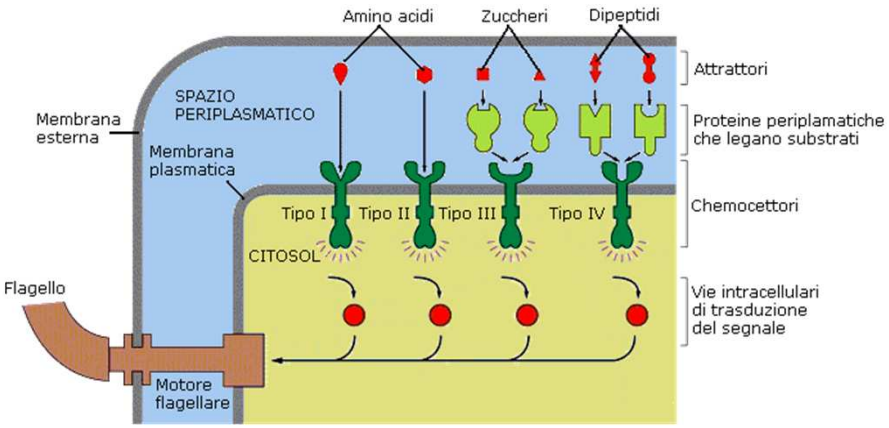


 Macnab R. M. *Annu. Rev. Microbiol.* 2003

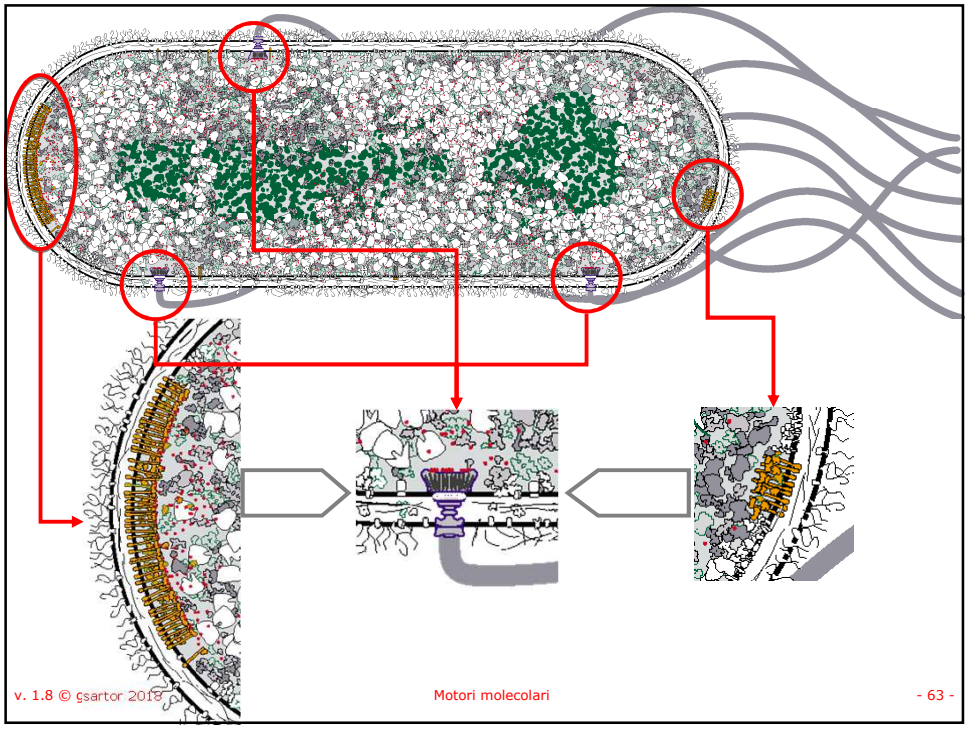


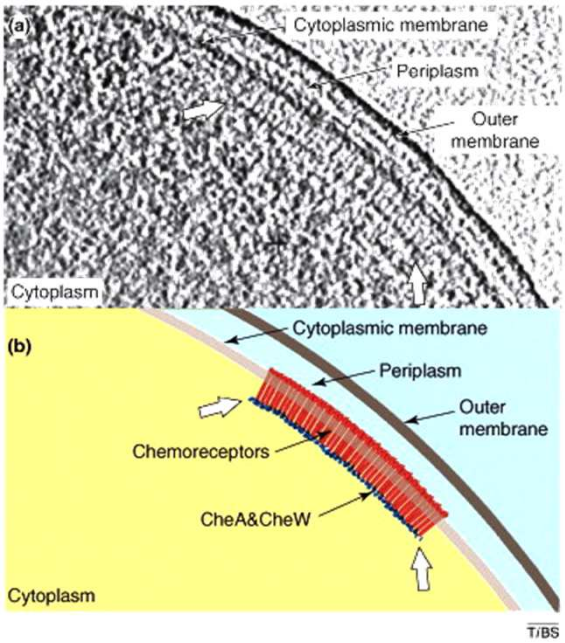
Parkinson *et al., Curr. Opin. Microbiol.* 2005

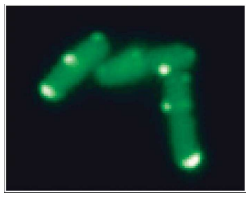
Differenti recettori



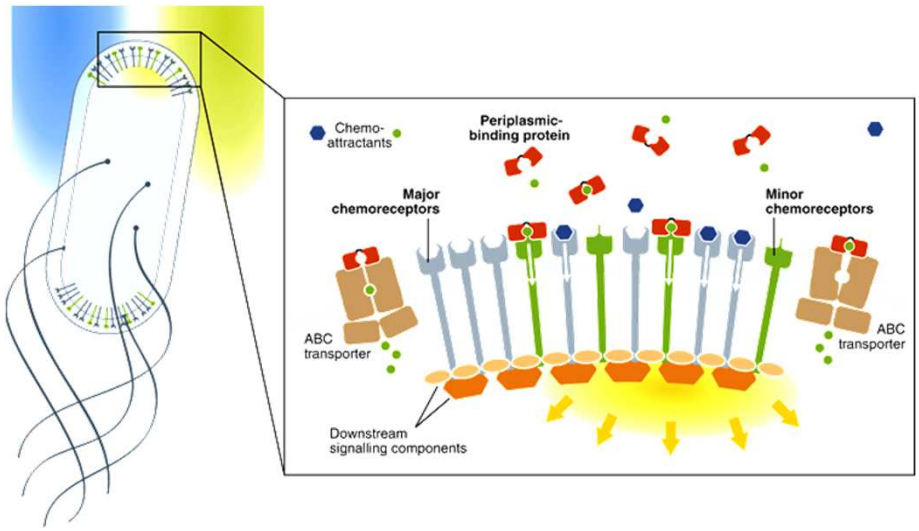
Raccolti ai poli della cellula




(a)
Cytoplasmic membrane
Periplasm
Outer membrane
Cytoplasm
(b)
Cytoplasmic membrane
Periplasm
Outer membrane
Chemoreceptors
CheA&CheW
Cytoplasm
TiBS

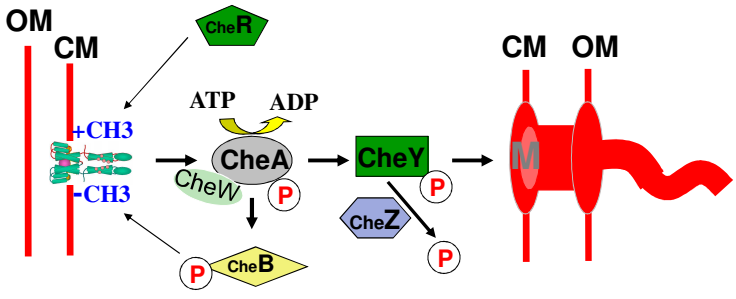


CheY::YFP

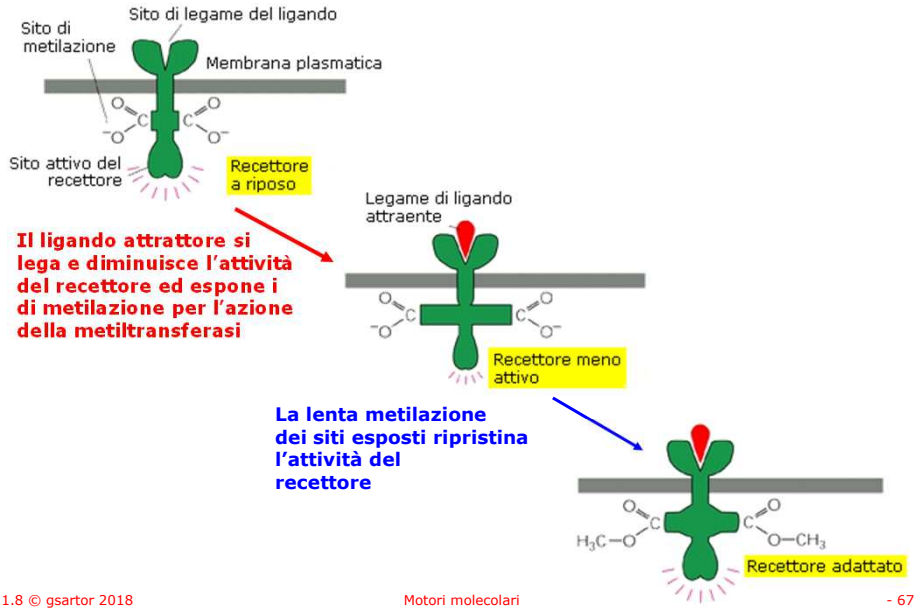


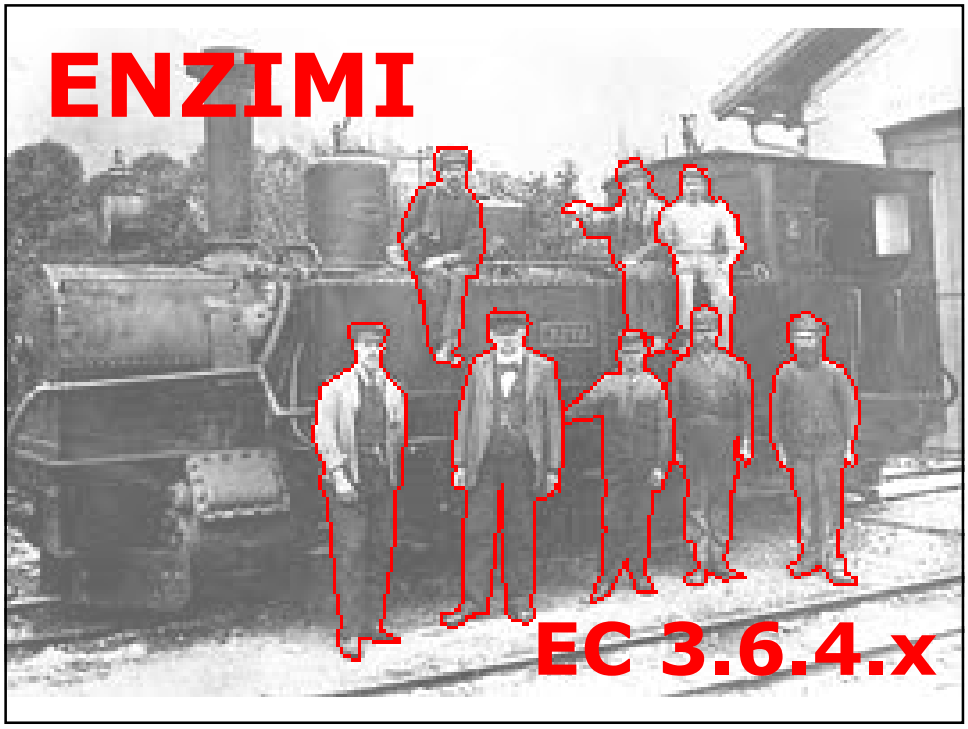
Underbakke & Kiessling , EMBO J. 2010

Adattamento via metilazione del recettore



Attivazione sequenziale e adattamento (via metilazione) di un chemocettore



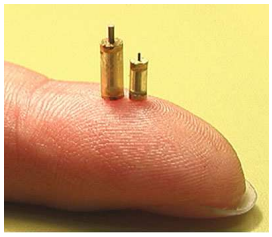


Acting on acid anhydrides to facilitate cellular and subcellular movement

- **EC 3.6.4.1 myosin ATPase**
- **EC 3.6.4.2 dynein ATPase**
- **EC 3.6.4.3 microtubule-severing ATPase**
- **EC 3.6.4.4 plus-end-directed kinesin ATPase**
- **EC 3.6.4.5 minus-end-directed kinesin ATPase**
- EC 3.6.4.6 vesicle-fusing ATPase
- EC 3.6.4.7 peroxisome-assembly ATPase
- EC 3.6.4.8 proteasome ATPase
- EC 3.6.4.9 chaperonin ATPase
- EC 3.6.4.10 non-chaperonin molecular chaperone ATPase
- EC 3.6.4.11 nucleoplasmin ATPase
- EC 3.6.4.12 DNA helicase
- EC 3.6.4.13 RNA helicase

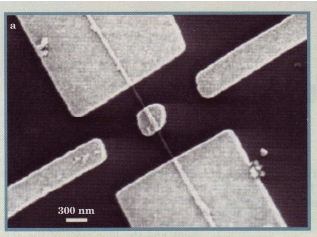
Motori molecolari

~ mm



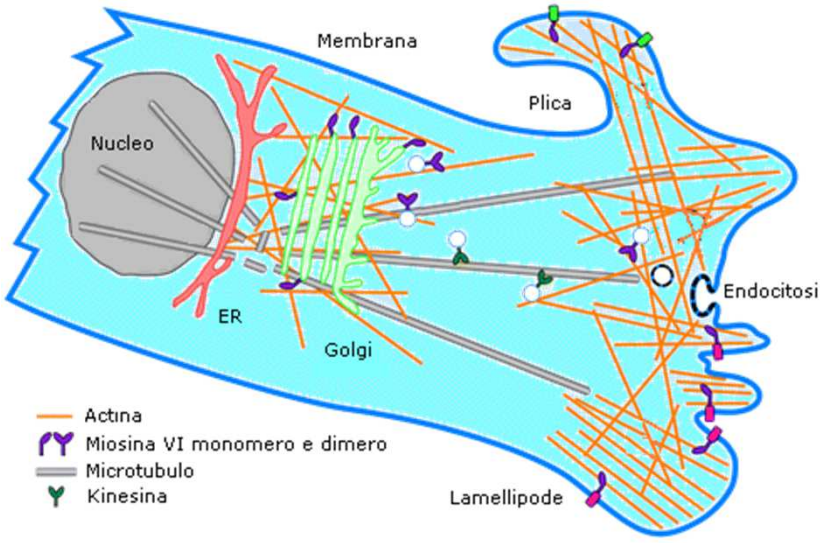
~ 10 mm

~ 100 nm



~ 10 nm

Figure 1. Nonequilibrium small systems. (a) In this scanning electron microscope image of an integrated synthetic actuator, the central metal-plate rotor is attached to a multiwalled carbon nanotube (MWNT) that acts as a support shaft. Electrical contact to the rotor plate is made via the MWNT and its anchor pads. A synchronized electrostatic force can induce rotary motion about the axis of the MWNT. (Adapted from ref. 16.) **(b)** This artist's rendition, based on crystallographic studies, shows a kinesin motor walking along a microtubule in a hand-over-hand (blue regions) fashion to carry organelles and other cargo from one part of the cell to another. Every step of the motor involves the hydrolytic conversion of chemical energy from adenosinetriphosphate into mechanical work. The reaction cycle is completed with the release of the hydrolysis product, adenosinediphosphate (ADP). Each of the motor's hands is about 5 nm long.



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  - NELSON David L. , COX Michael M. I PRINCIPI DI BIOCHIMICA DI LEHNINGER - Zanichelli
  - GARRETT Reginald H., GRISHAM Charles M. BIOCHIMICA con aspetti molecolari della Biologia cellulare - Zanichelli
  - VOET Donald , VOET Judith G , PRATT Charlotte W FONDAMENTI DI BIOCHIMICA [ISBN 978-8808-06879-8] - Zanichelli

- E dalla consultazione di svariate risorse in rete, tra le quali:
  - Kegg: Kyoto Encyclopedia of Genes and Genomes http://www.genome.ad.jp/kegg/
  - Brenda: http://www.brenda.uni-koeln.de/
  - Protein Data Bank: http://www.rcsb.org/pdb/
  - Per il reperimento di alcune figure devo ringraziare la Studentessa (ora Dottoressa) Alessandra Ricotta.
  - Il materiale è stato inoltre rivisto e corretto dalla **Prof. Giancarla Orlandini** dell'Università di Parma alla quale va il mio sentito ringraziamento.

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