

1 2020年度・M1セミナー

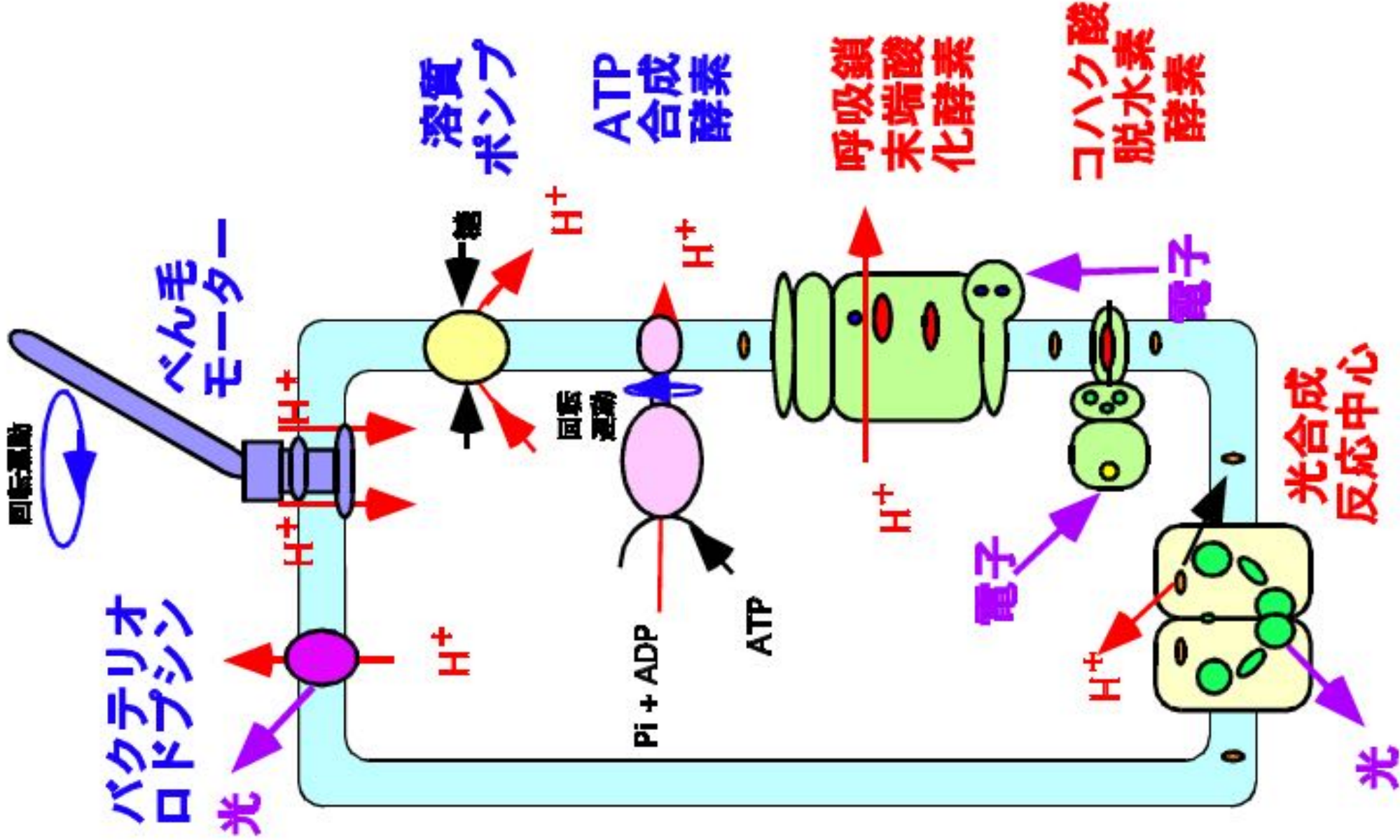
大学院理学研究科・生命理学専攻
超分子機能学講座：生体膜機能グループ



細菌から切り開く 生命の基本原理

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（アイソトープセンター）

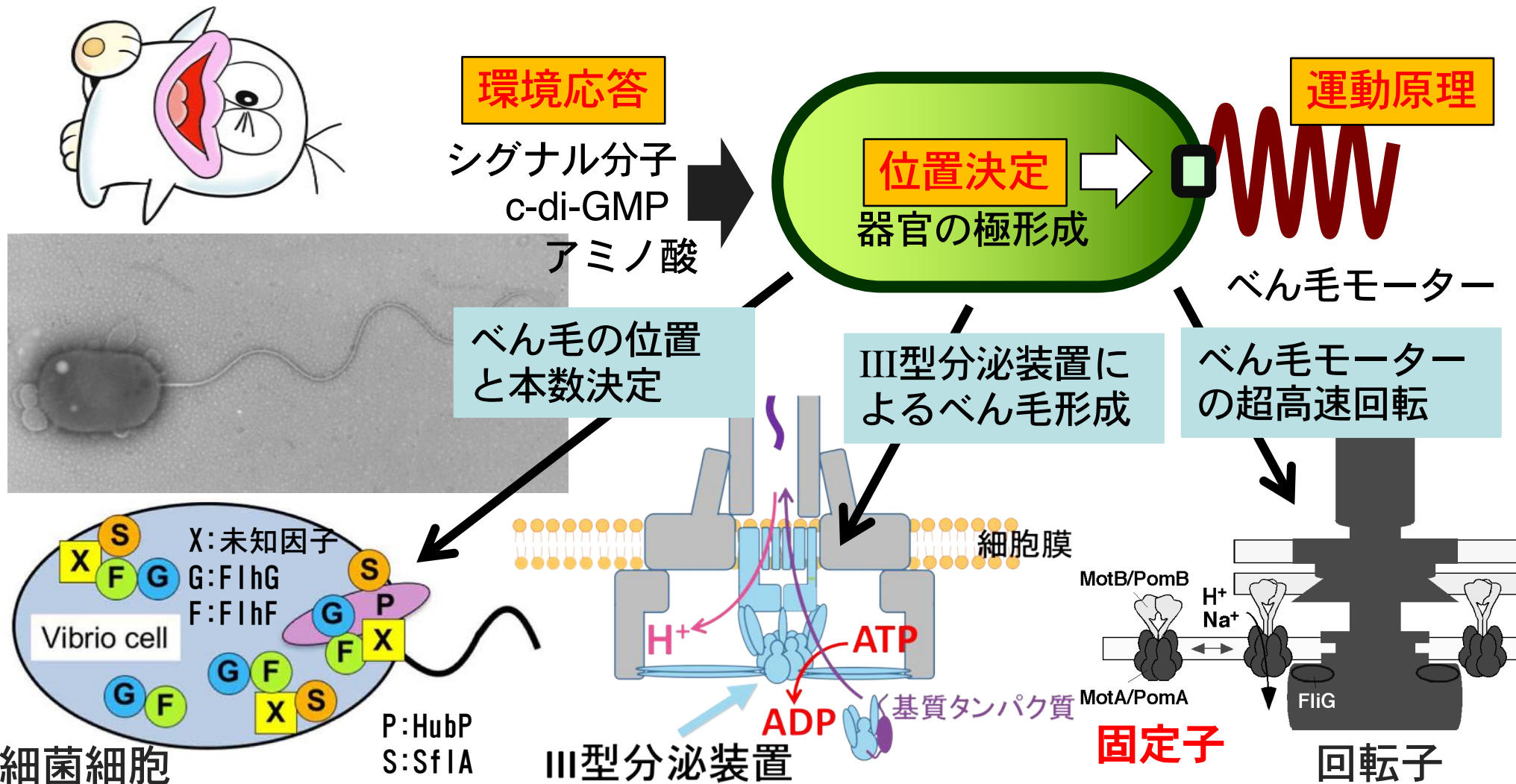
2 膜で機能する蛋白質達



3 細菌から切り開く生命の基本原理

どのようにして環境に応答し運動を制御し、運動器官を適切な位置に構築してエネルギー変換し、運動をするのだろうか？

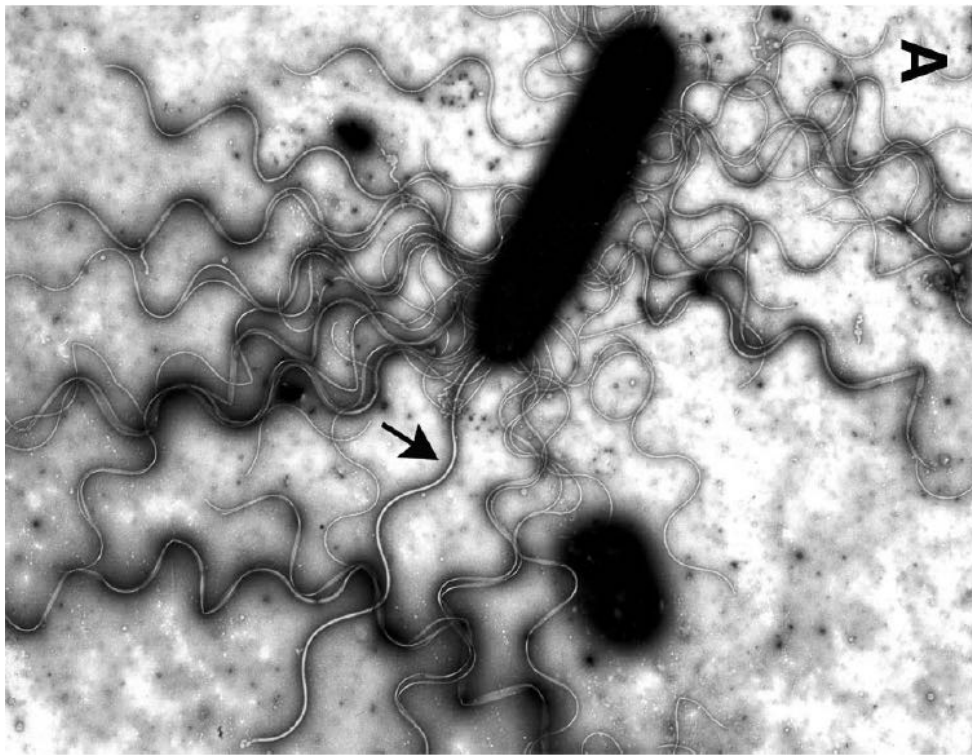
基本生命現象は細菌べん毛をアウトプットとして解析できる





イオン流を回転エネルギーに変換する様子アニメーション
エネルギー変換装置の構造ダイナミクスと反応の共
役についてはこのアニメーションは答えていない。

Lateral and polar flagella of *Vibrio*



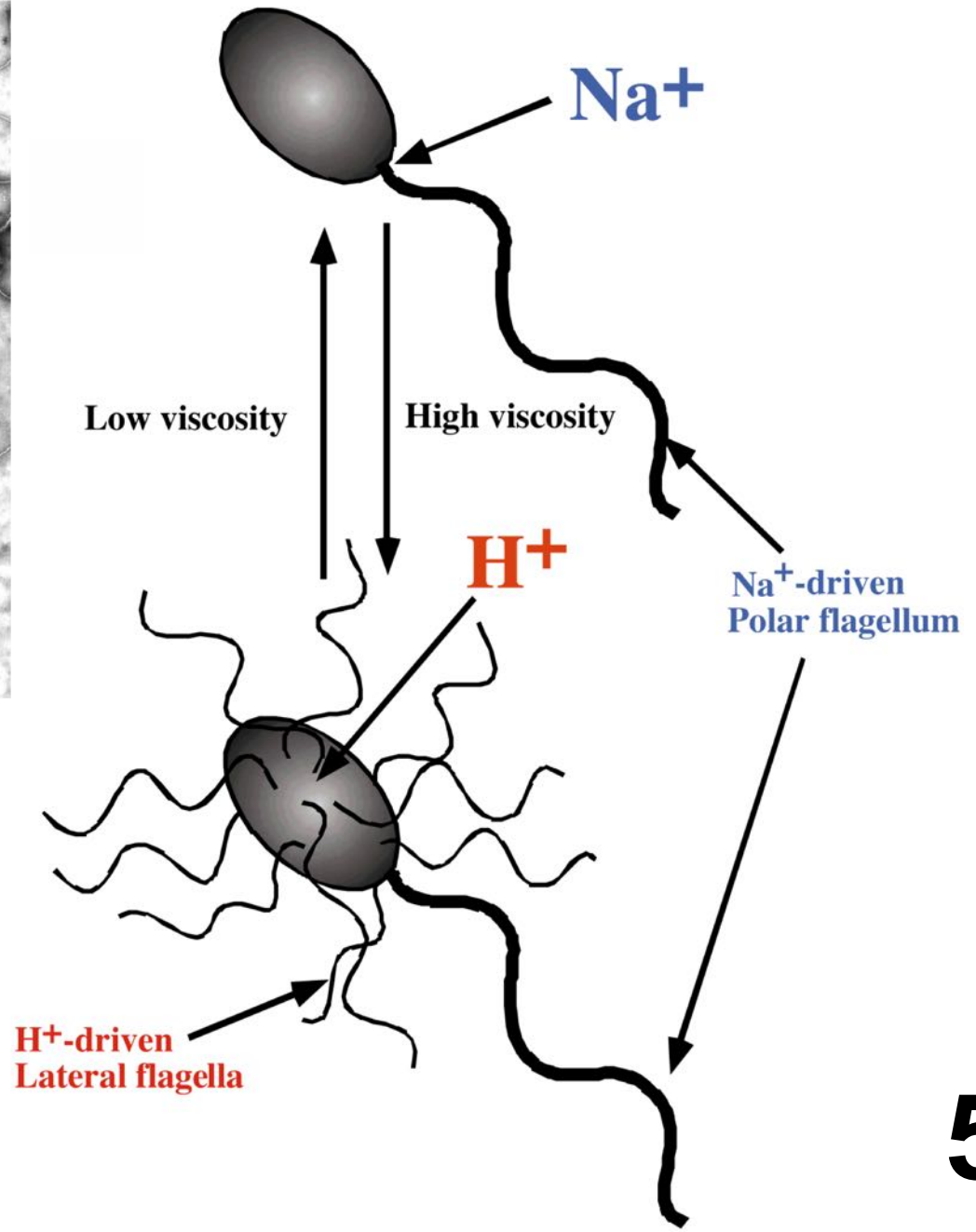
Nature, v.355, p 185- (1992)

Polar and lateral flagellar motors of marine *Vibrio* are driven by different ion-motive forces

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First paper of sodium-driven motor genes

JOURNAL OF BACTERIOLOGY, Aug. 1997, p. 5104–5110
0021-9193/97/\$04.00+0
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Putative Channel Components for the Fast-Rotating Sodium-Driven Flagellar Motor of a Marine Bacterium

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AND MICHIO HOMMA*

Division of Biological Science, Graduate School of Science, Nagoya University, Chikusa-Ku, Nagoya 464-01, Japan

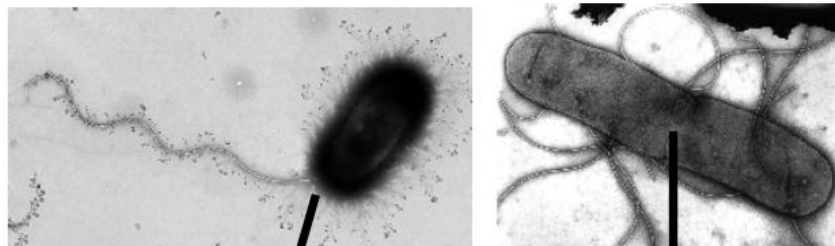
Received 8 April 1997/Accepted 13 June 1997

The polar flagellum of *Vibrio alginolyticus* rotates remarkably fast (up to 1,700 revolutions per second) by using a motor driven by sodium ions. Two genes, *motX* and *motY*, for the sodium-driven flagellar motor have been identified in marine bacteria, *Vibrio parahaemolyticus* and *V. alginolyticus*. They have no similarity to the genes for proton-driven motors, *motA* and *motB*, whose products constitute a proton channel. MotX was proposed to be a component of a sodium channel. Here we identified additional sodium motor genes, *pomA* and *pomB*, in *V. alginolyticus*. Unexpectedly, PomA and PomB have similarities to MotA and MotB, respectively, especially in the predicted transmembrane regions. These results suggest that PomA and PomB may be sodium-conducting channel components of the sodium-driven motor and that the motor part consists of the products of at least four genes, *pomA*, *pomB*, *motX*, and *motY*. Furthermore, swimming speed was controlled by the expression level of the *pomA* gene, suggesting that newly synthesized PomA proteins, which are components of a force-generating unit, were successively integrated into the defective motor complexes. These findings imply that Na⁺-driven flagellar motors may have similar structure and function as proton-driven motors, but with some interesting differences as well, and it is possible to compare and study the coupling mechanisms of the sodium and proton ion flux for the force generation.

Flagellar basal body and rotary motor

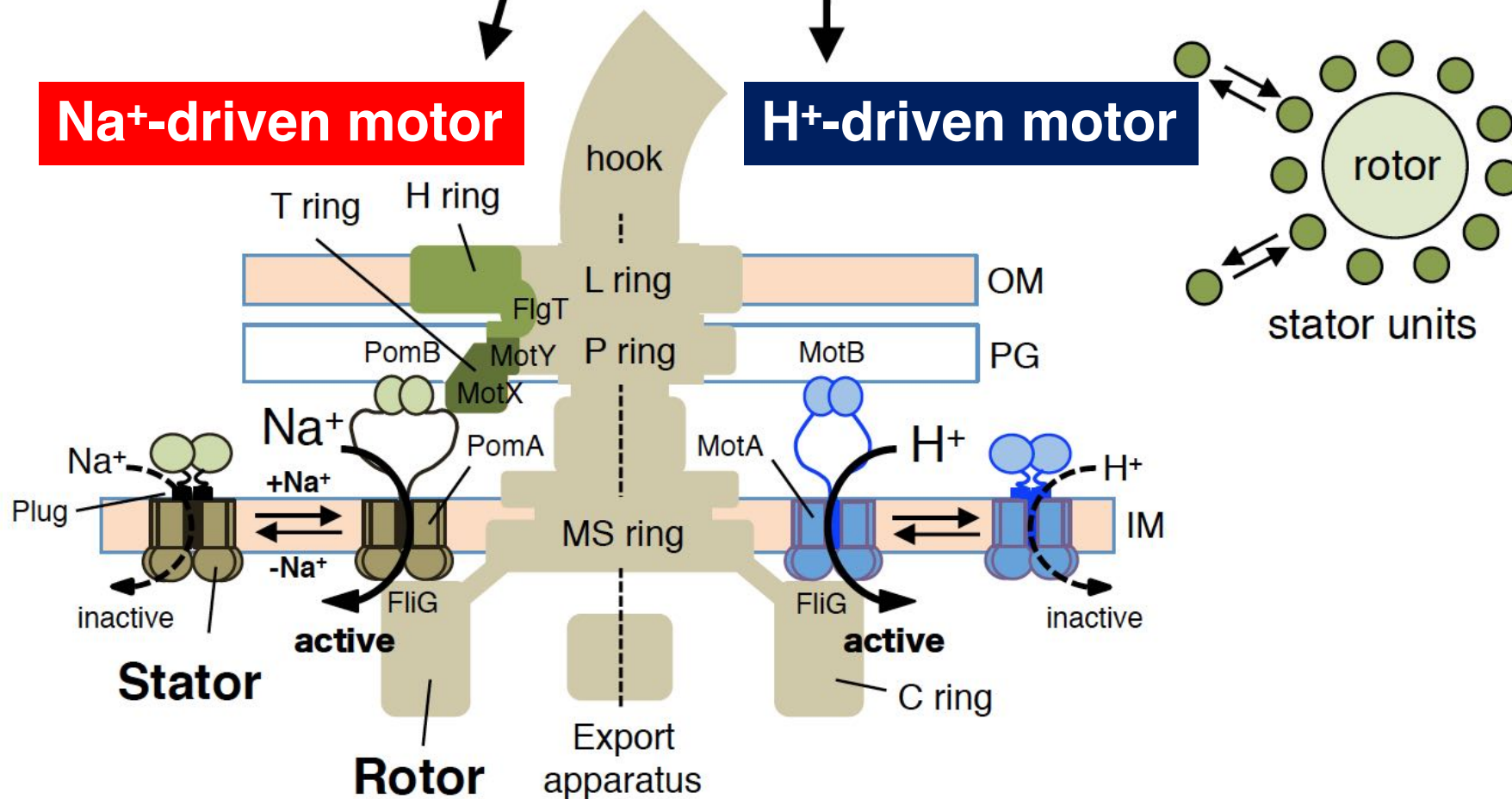
Vibrio alginolyticus

Salmonella enterica

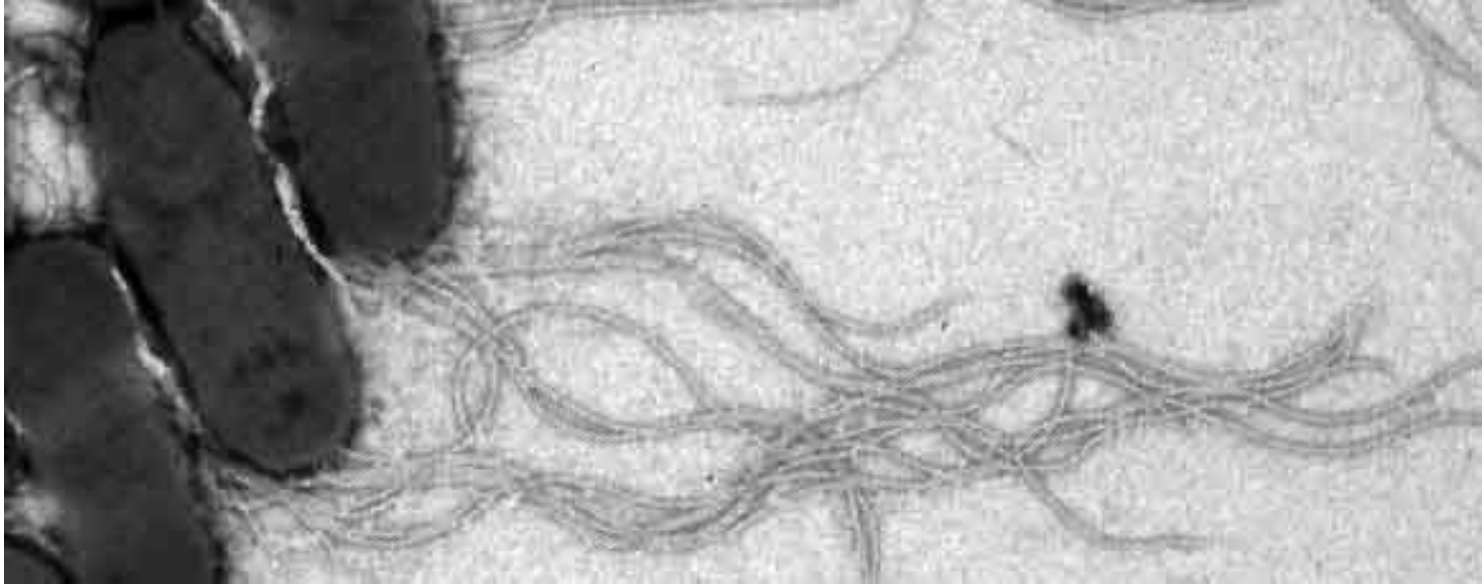


Na⁺-driven motor

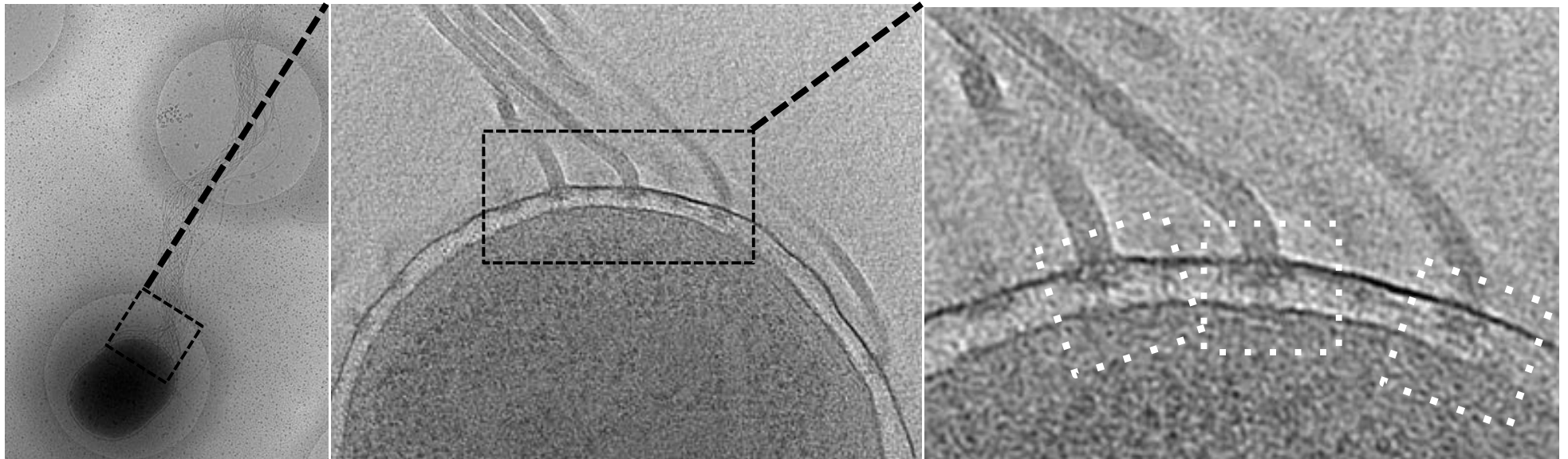
H⁺-driven motor



8 Multiflagellar phenotype (KK148) of *V. alginolytius*



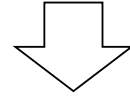
Negative staining



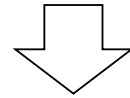
Cryo EM

Zhu et al., PNAS (2017)

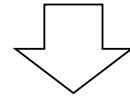
Bacterial culture preparation



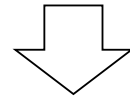
Cryo-ET sample preparation



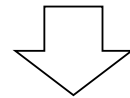
Cryo-ET data acquisition



Cryo-ET data processing
(Alignment & 3D reconstruction)

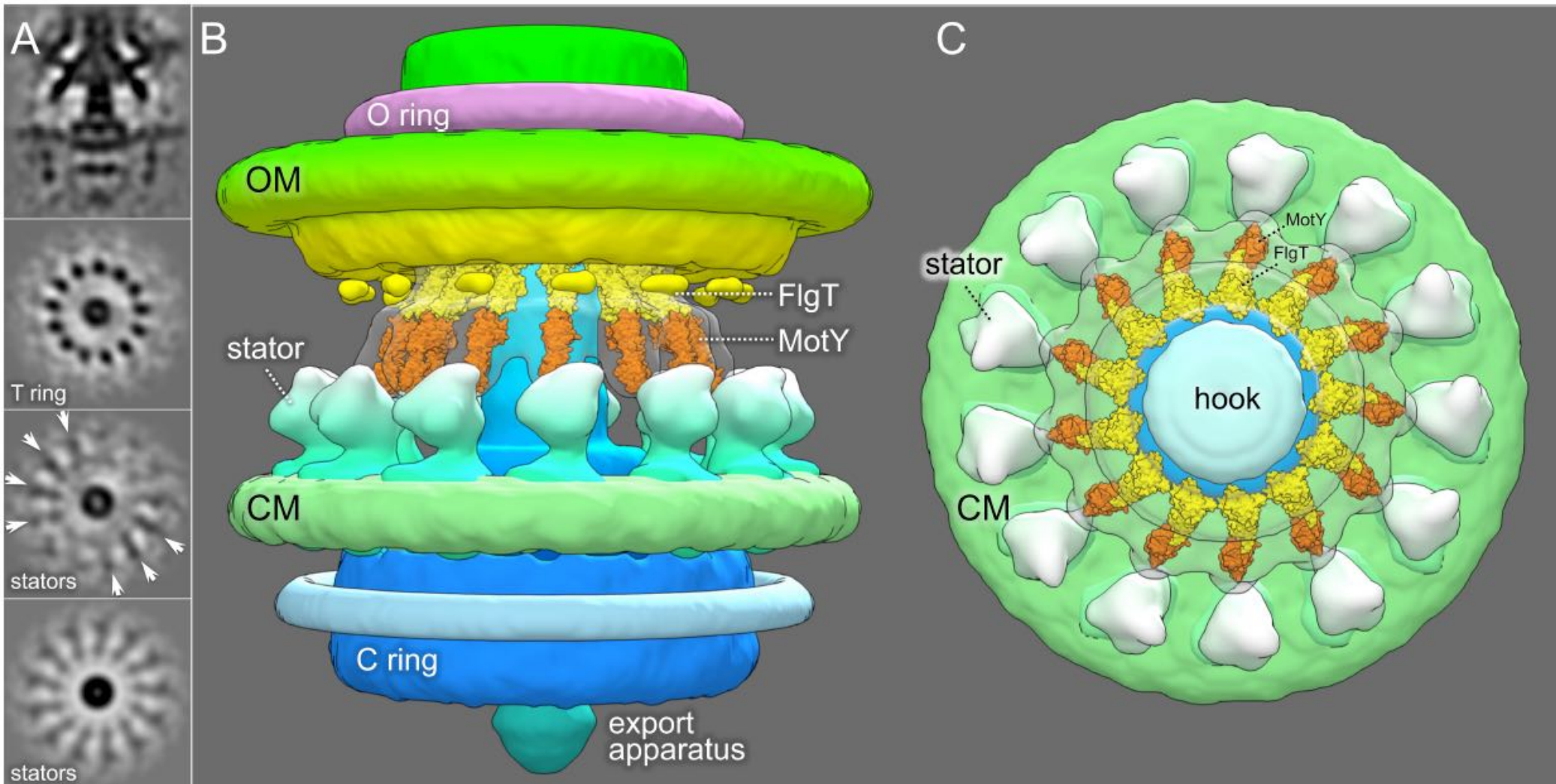


Sub-tomogram analysis
(Classification & Averaging)



3D visualization
(“Mapback” & Segmentation)

Characterization of T ring composed of MotX and MotY



13 fold symmetry of T ring

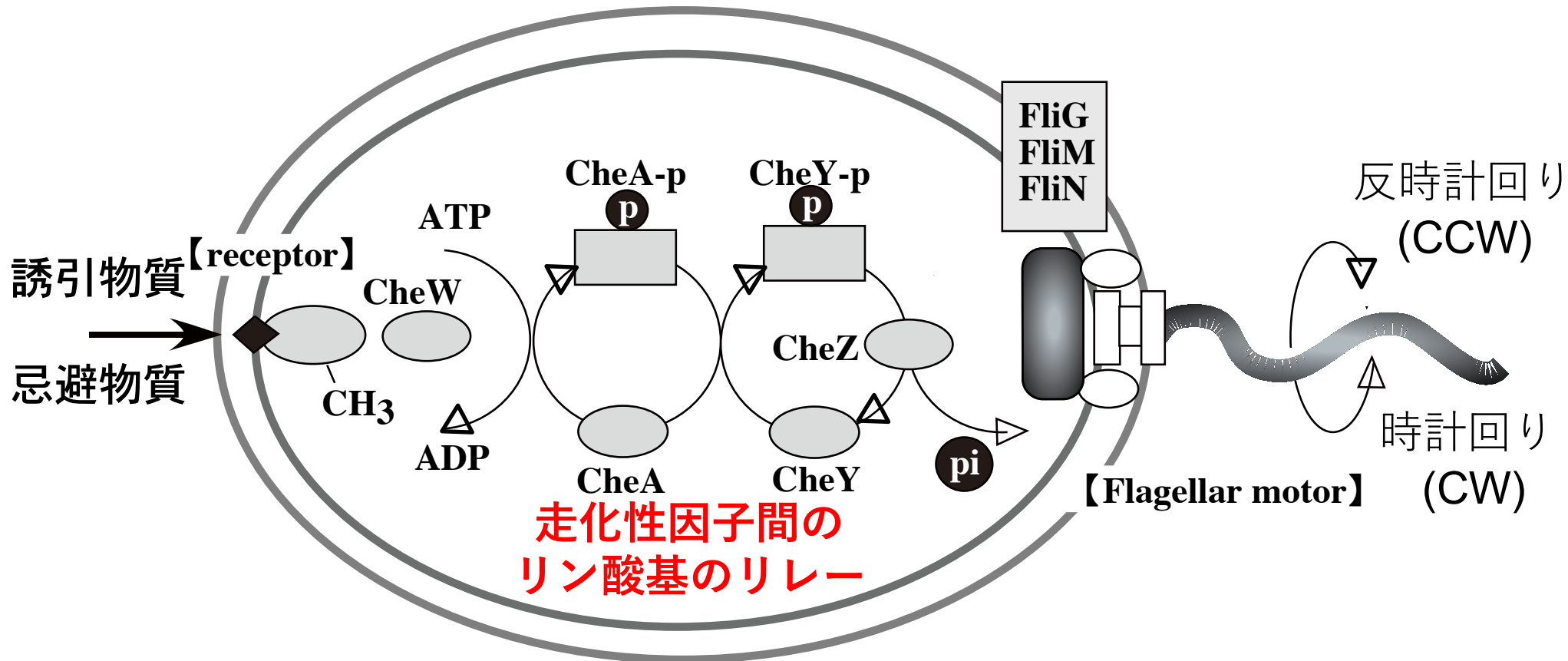
細菌が誘引物質（セリン）に集まる様子



走化性シグナルによるべん毛の回転方向制御

CheY欠損ではべん毛はCCWのみで回転する

走化性シグナルによるべん毛回転方向制御の模式図

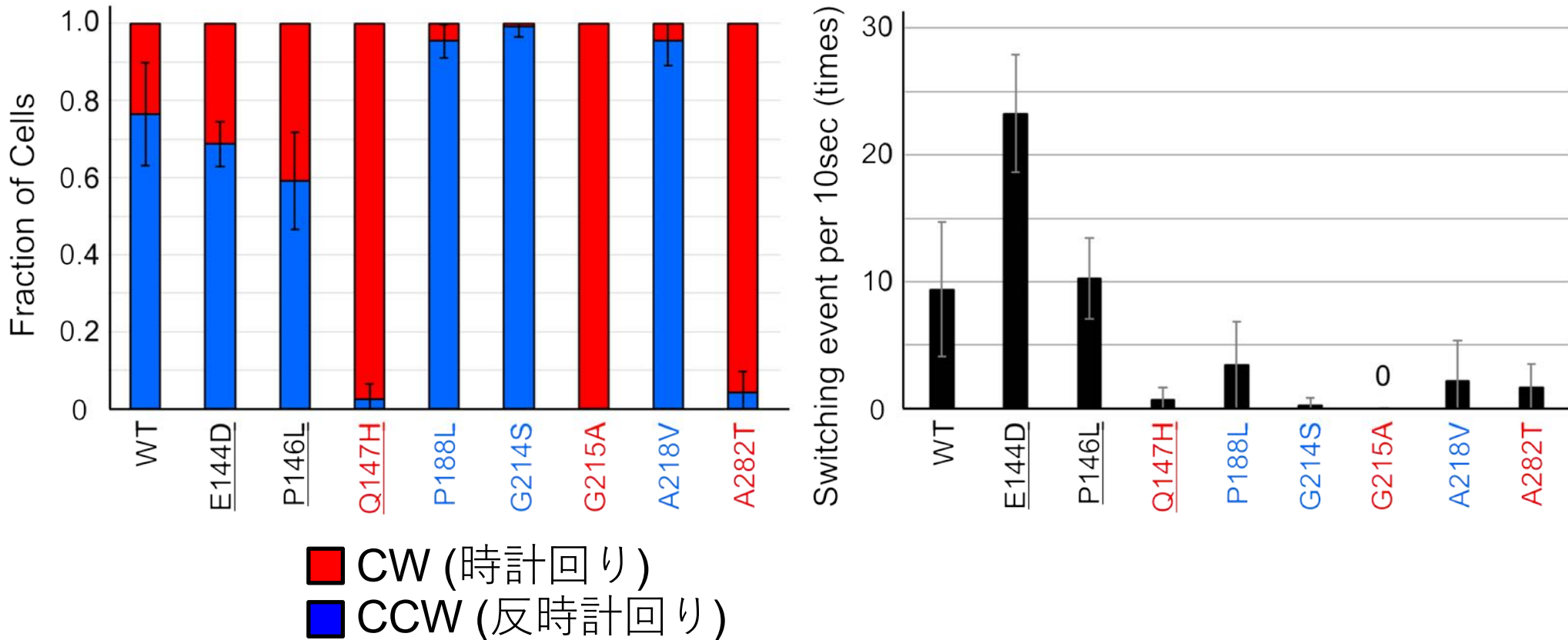


CheYとFliMの結合が回転方向を決定する

リン酸化CheY(CheY-P)が回転子中のFliMと結合：CW回転

CheY-Pが脱リン酸化されFliMから解離：CCW回転

回転方向制御が異常になる *fliG* 変異体

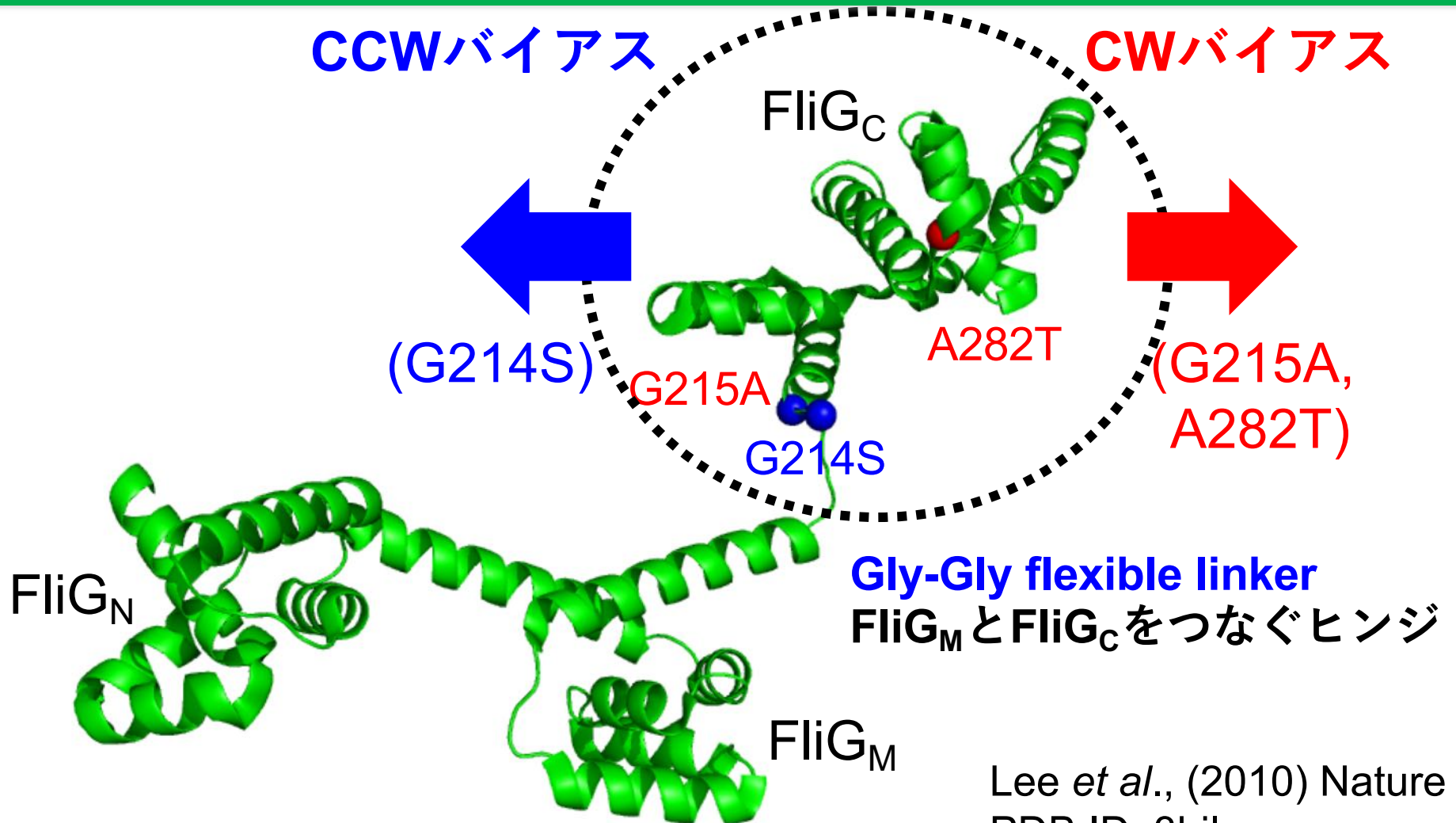


回転方向制御が異常になる **FliG** 変異体の取得

- CWへの回転の偏り (CW-biased) : Q147H, G215A, A282T
- CCWへの回転の偏り (CCW-biased) : P188L, G214S, A218V

(Nishikino *et al.*, 2016)

G214S, G215A, A282T変異によるFliG_Cの構造変化

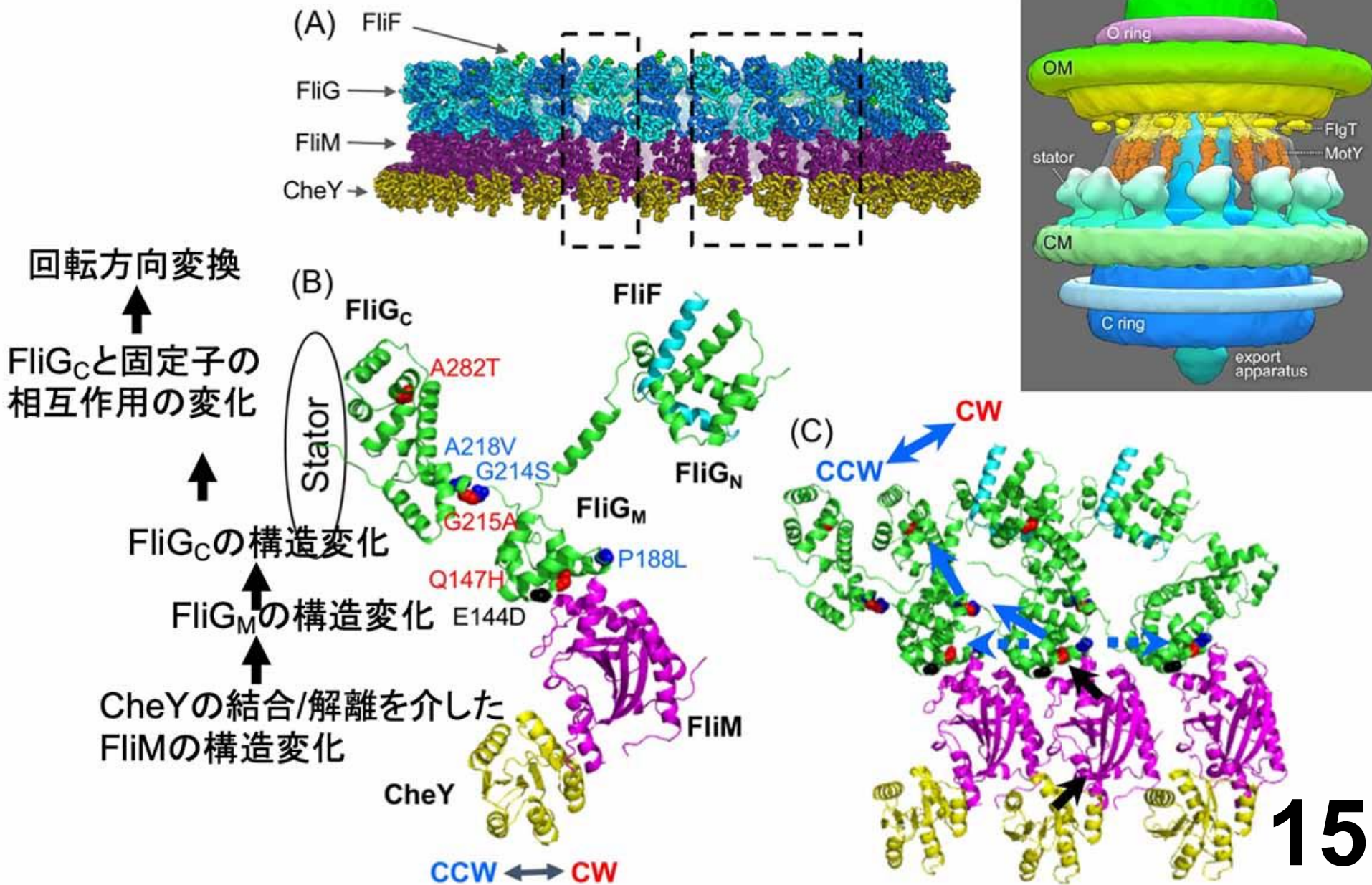


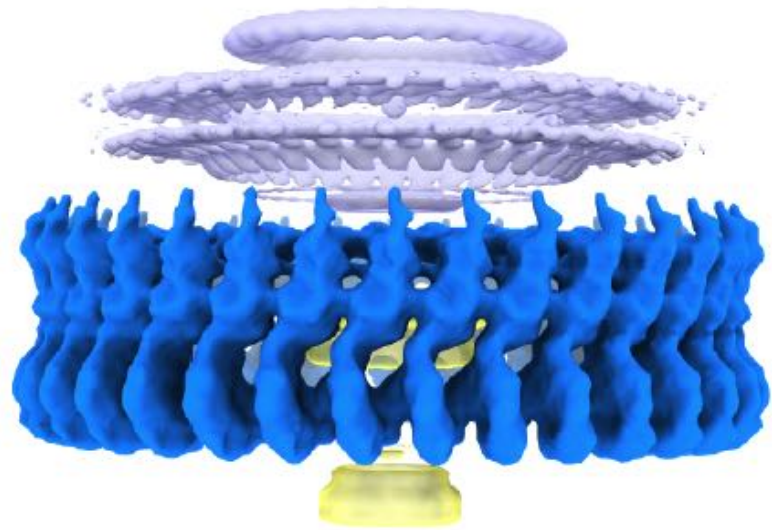
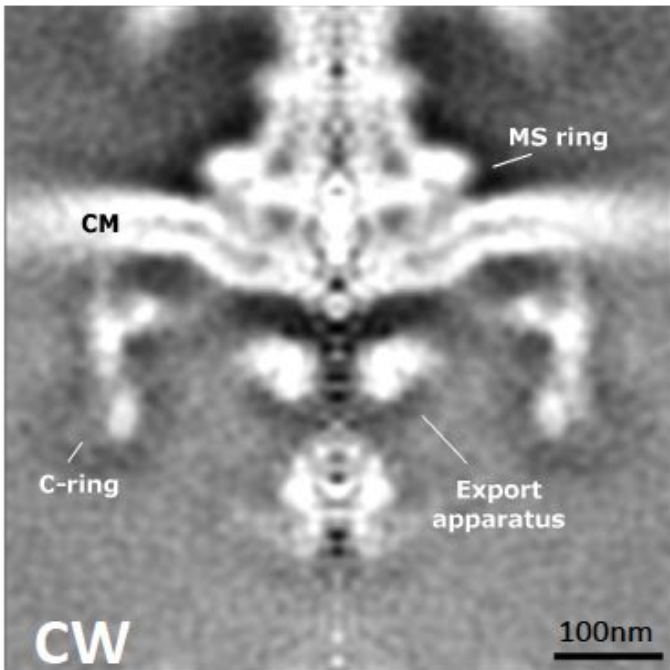
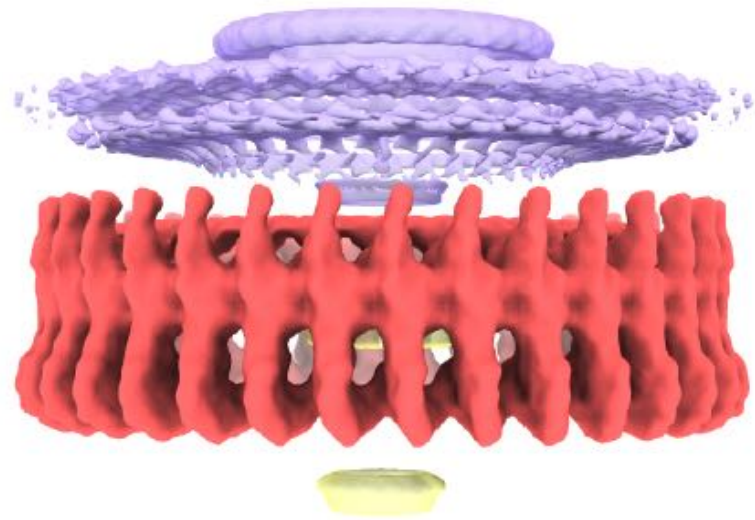
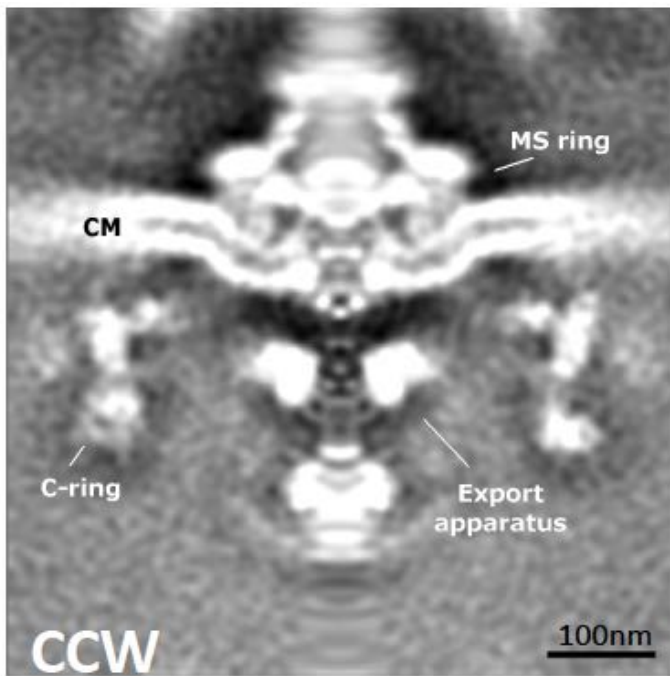
Lee *et al.*, (2010) Nature
PDB ID: 3hjl
Aquifex aeolicus

FliG_Cのコンフォメーション変化が
回転方向を決定する

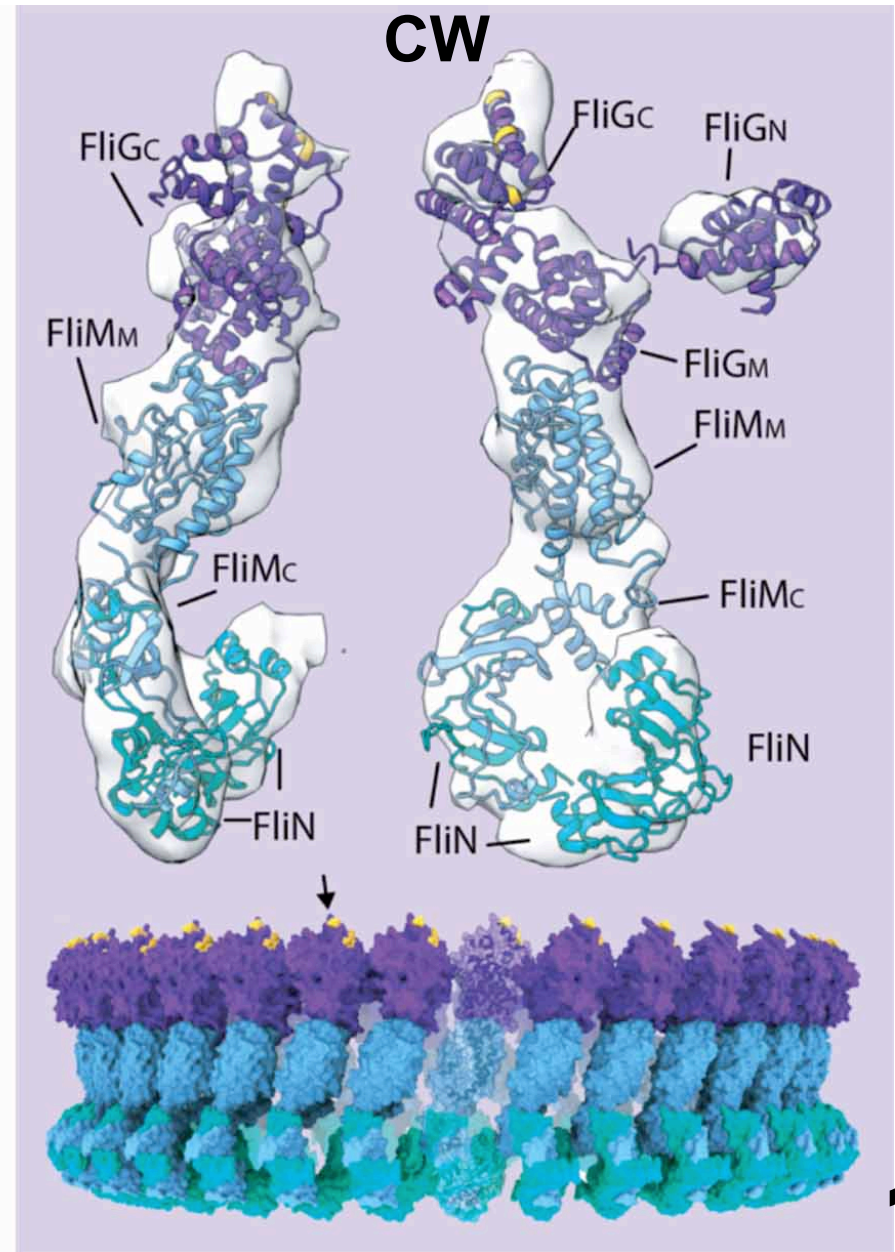
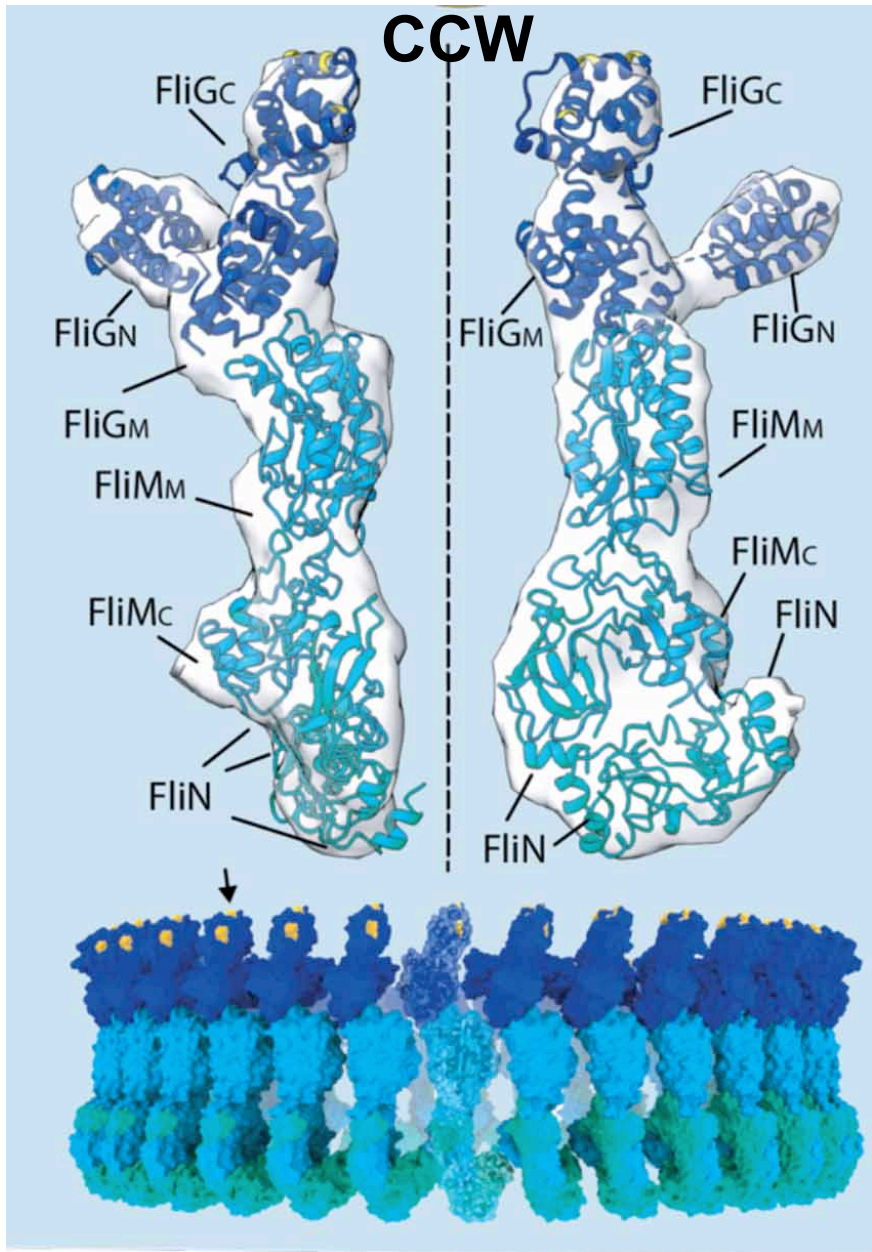
(Nishikino *et al.*, 2016, Miyanoiri *et al.*, 2017)

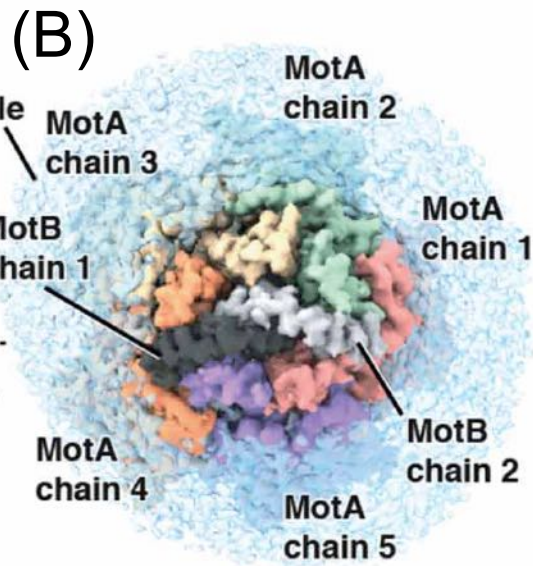
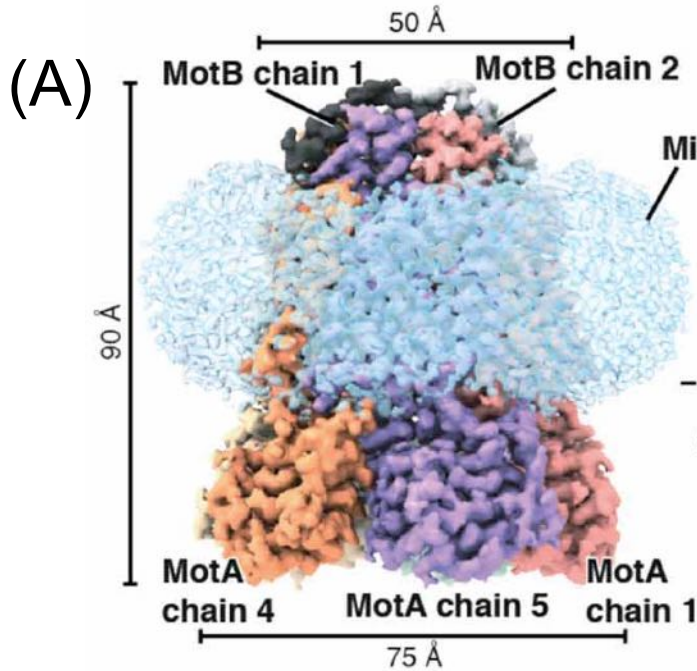
べん毛モーター回転方向転換のクラッチ分子機構





CW-CCW回転時のFliG・FliM・FliN複合体の構造モデル





Campylobacter jejuni

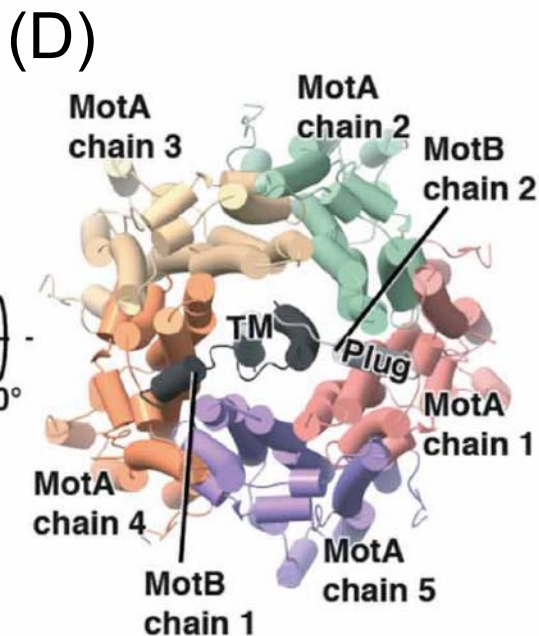
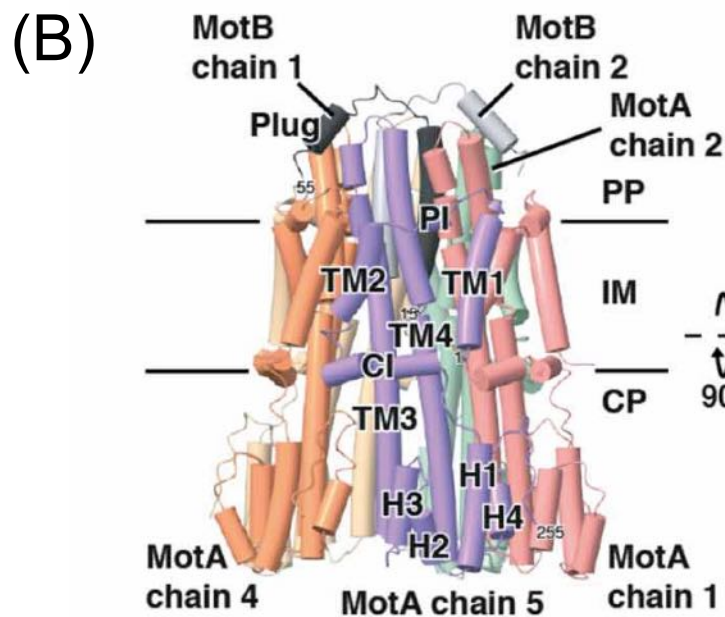
登録2020年5月15日

Structure and function of stator units of the bacterial flagellar motor, bioRxiv, Santiveri *et al.*,

Clostridium sporogenes
Bacillus subtilis

登録2020年5月13日

Structures of the stator complex that drives rotation of the bacterial flagellum, bioRxiv, Deme *et al.*,





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エネルギー変換装置の構造ダイナミクスと反応の共
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Thank you for your attention.

Homma

