College de France _ 27 janvier 2016

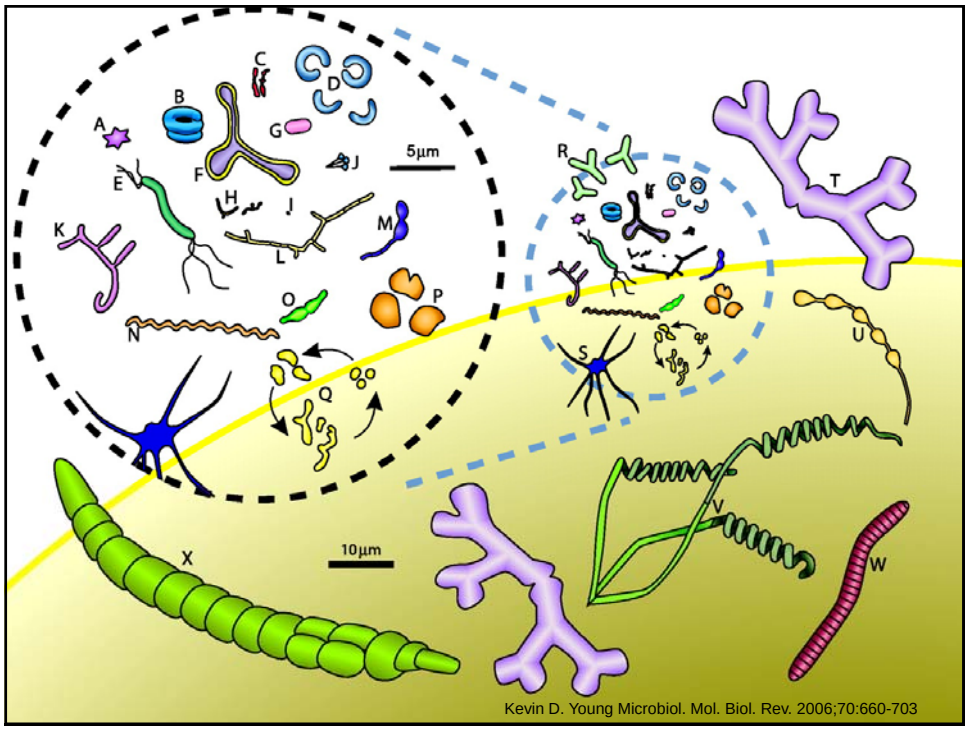
Comment les bactéries déterminent leur forme

une cible pour de nouveaux antibiotiques ?

Rut CARBALLIDO LOPEZ
Prokaryotic Cell Development Lab

INRA SCIENCE & IMPACT Micalis

2 µm



Kevin D. Young Microbiol. Mol. Biol. Rev. 2006;70:660-703

Variety of prokaryotic cell shapes

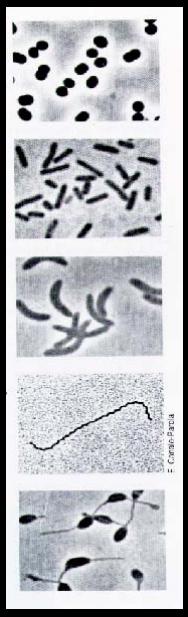
Coccus

Rod

Spirillum

Spirochete

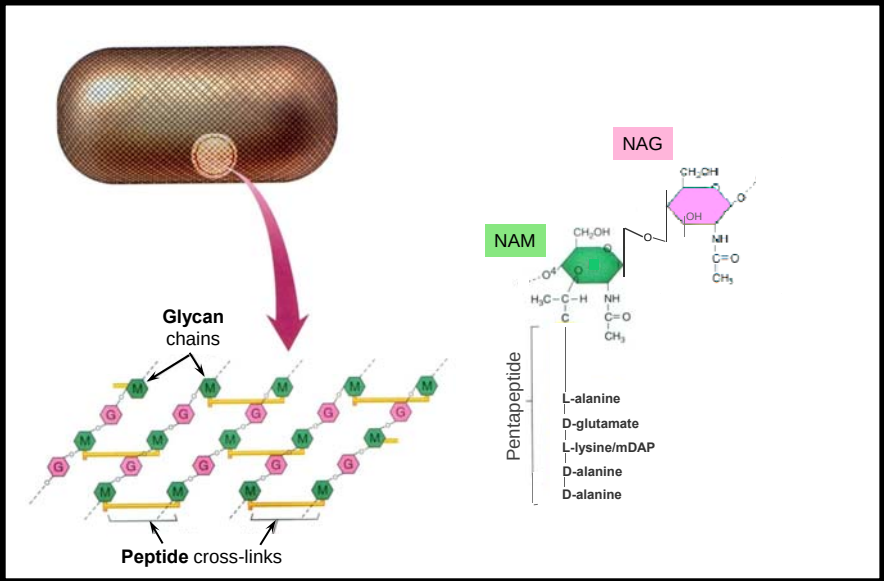
Budding and appendaged bacteria

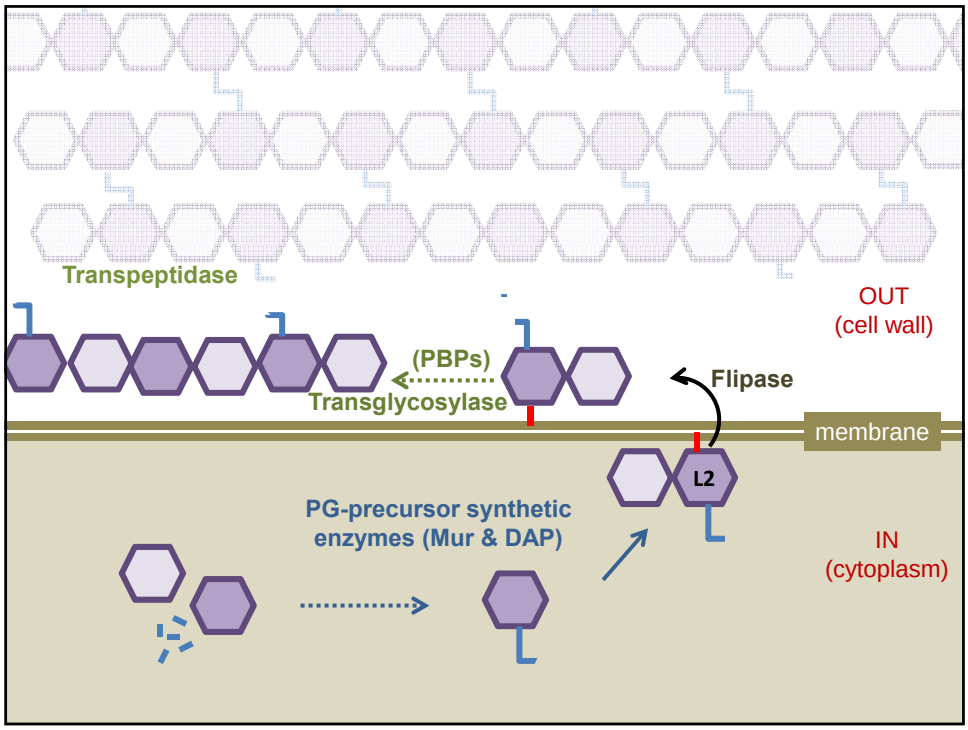


"... Space must be cut off, shaped, defined, for us to inhabit.
From cradle to coffin, it's enclosure that defines us"

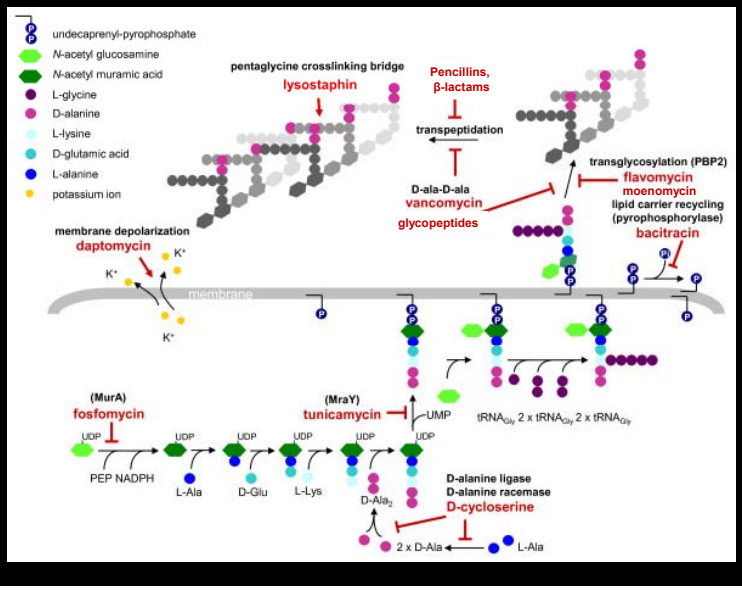
– Robert Morgan (1992)

Cell Wall Peptidoglycan



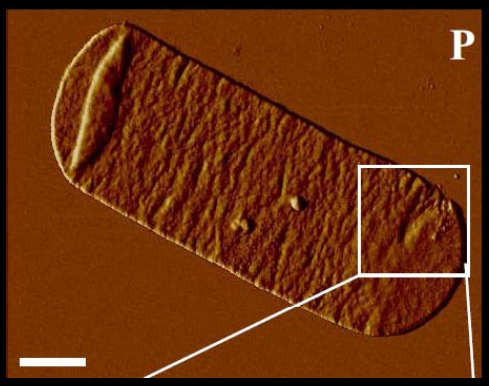


The enzymatic steps involved in cell wall synthesis are the targets of cell wall antibiotics.



Dengler et al. BMC Microbiology2011

The Cell Wall is a major determinant of shape



B. subtilis sacculus (AFM)
(Hayhurst, *et al.* PNAS 2008)

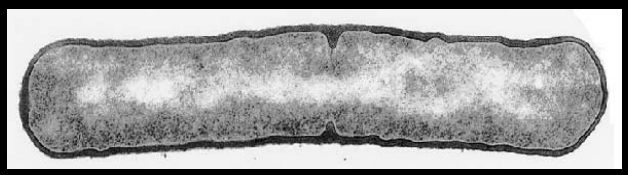
Isolated cell wall sacculi retain bacterial cell shape

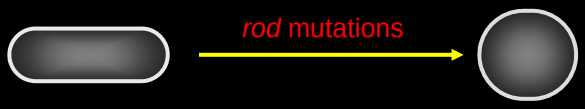
The Cell Wall is a major determinant of shape

© Arnaud Chastanet

Removal of the cell wall produces spherical cells (protoplasts)

Classical genetics of cell shape in rod-shaped bacteria



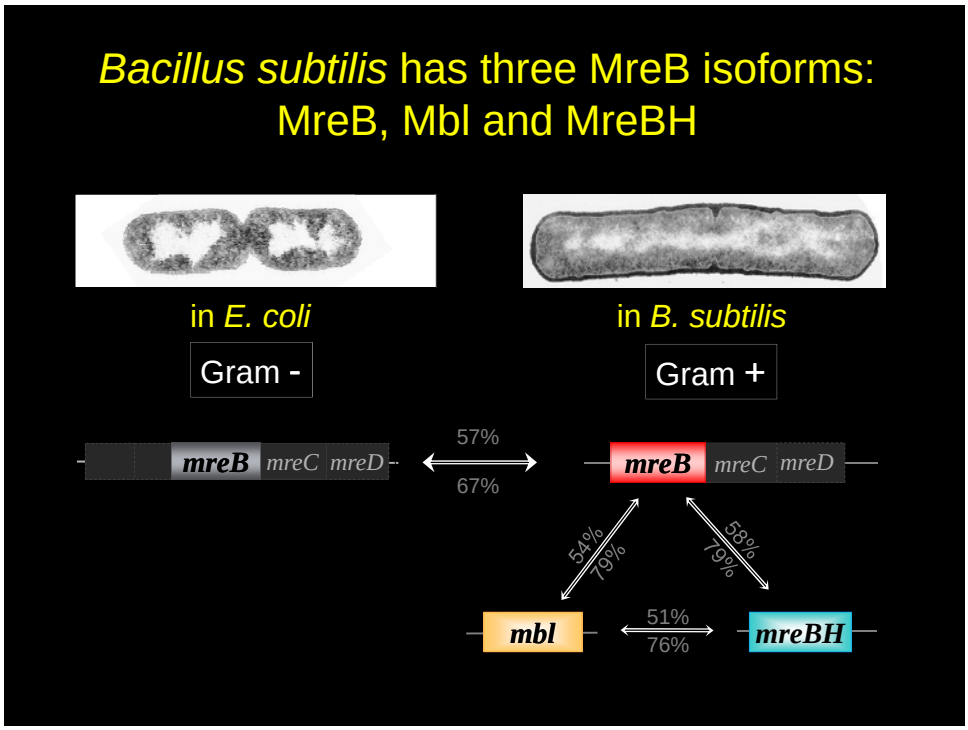


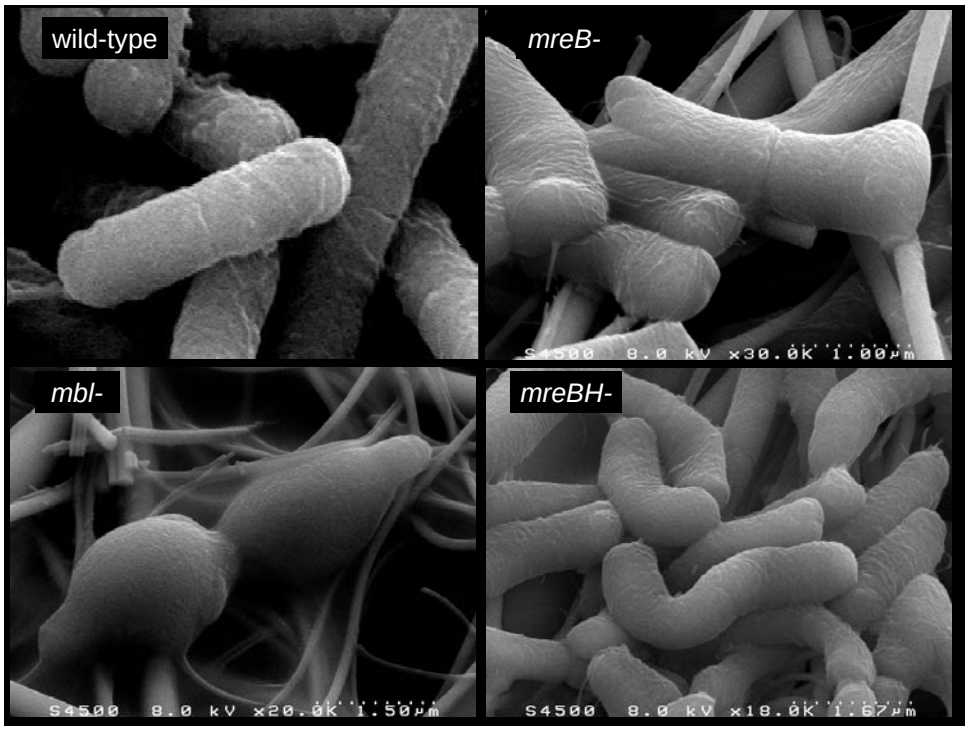
- *rodA* Peptidoglycan (wall) synthesis *rod*A, *pbpA*
- *rodC* Teichoic Acid (wall) synthesis *tagF*
- *rodB* Function unknown *mreBCD*

Bacteria with complex shapes tend to contain MreB-like proteins

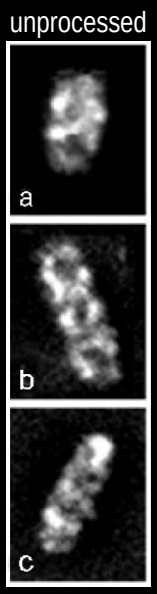
	Nr. organisms	example/group	Shape
mreB present	*8 / 2**	*B. subtilis* (3 genes), *E. coli*,	Rod
	3	*Vibrio cholera, Caulobacter crescentus*	Curved rod
	1	*Streptomyces coelicolor* (3 genes)	Filamentous
	5	*Helicobacter pylori, Borrelia burgdorferi*	Helical
	1	*Chlamydia* (2 species)	Pleiomorphic
mreB absent	*5 / 3**	*Strep. pneumoniae, Staph. aureus, Methanococcus jannaschii**	Round
	2	*Mycoplasma* (2 species)	Pleiomorphic

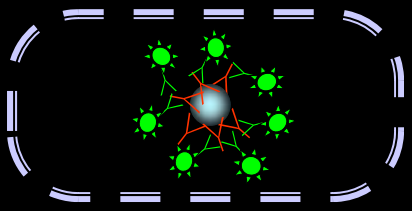
* Archaebacterium


Bacillus subtilis has three MreB isoforms:
MreB, Mbl and MreBH
in E. coli
Gram -
in B. subtilis
Gram +
mreB
mreC
mreD
57%
67%
mreB
mreC
mreD
54%
79%
58%
79%
mbl
51%
76%
mreBH


wild-type
mreB-
mbl-
mreBH-

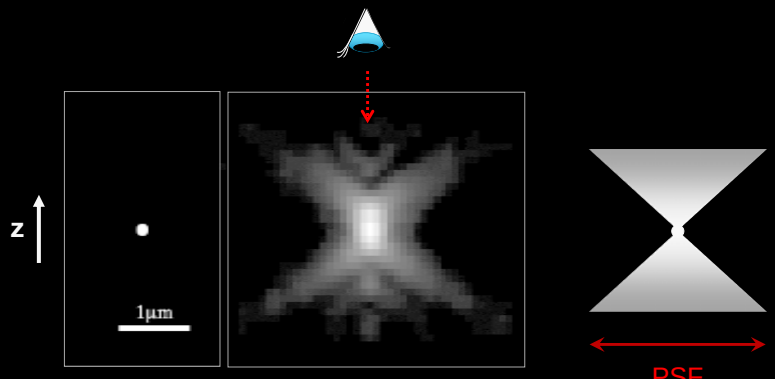
Subcellular localization of MreBs by IFM (Immunofluorescence Microscopy)





Indirect immunofluorescence

The 'Point Spread Function' (PSF)



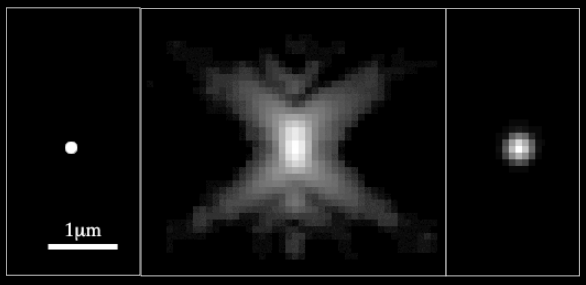
bead
175 nm
(diameter)

acquired image
(1.4NA/100 x Zeiss
Plan/Apo/Widefield)

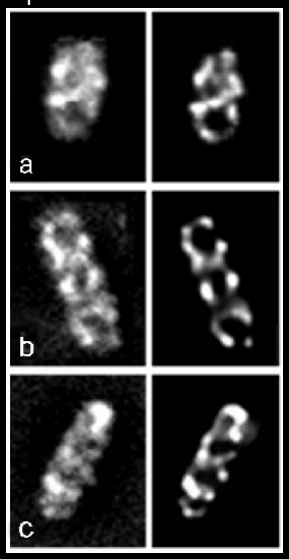
3D blurry image
of such a single
point light source

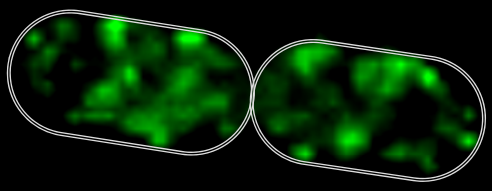
Deconvolution

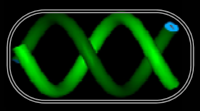
Mathematical operation used in image analysis to remove out-of-focus light above and below the focal plane (PSF)



drawn to scale | acquired image under the microscope | deconvolved image



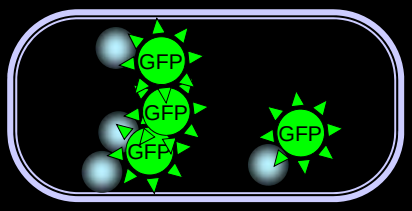




'MreB proteins form filamentous helical structures that encircle the cell'

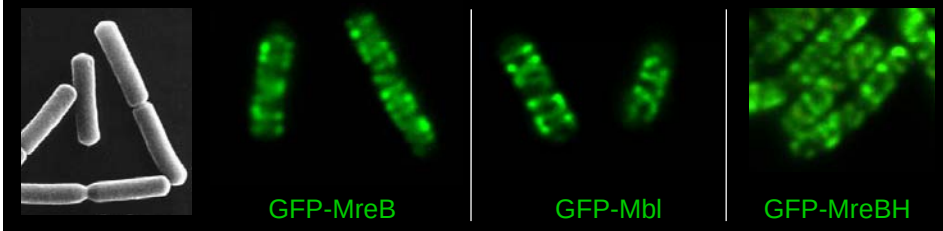
[Jones, Carballido-López & Errington (2001) ***Cell***]

Subcellular localization of GFP-MreBs fusions imaged by epifluorescence in live cells

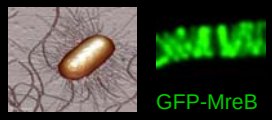


Subcellular localization of GFP-MreBs fusions imaged by conventional epifluorescence

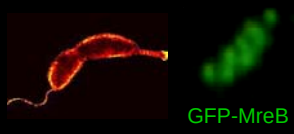
Bacillus subtilis



Escherichia coli

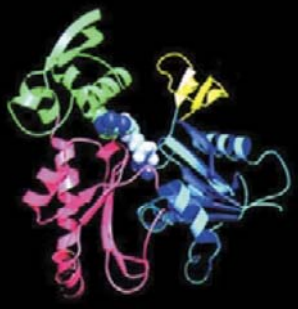


Caulobacter crescentus



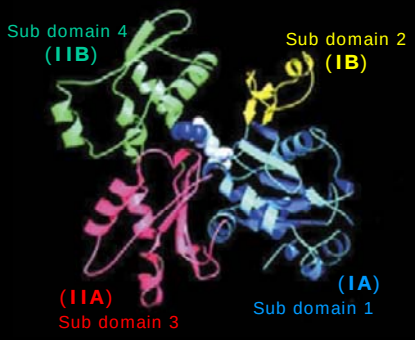
MreB proteins are structural homologues of eukaryotic actin

MreB (*T. maritima*)

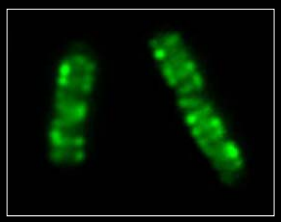


[van den Ent F, Amos LA, Lowe J. *Nature* 2001, 413:39-44]

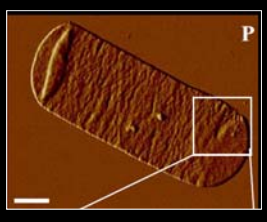
Eukaryotic ACTIN



Actin-like MreB proteins and the peptidoglycan celll wall are major determinants of shape in non-spherical bacteria



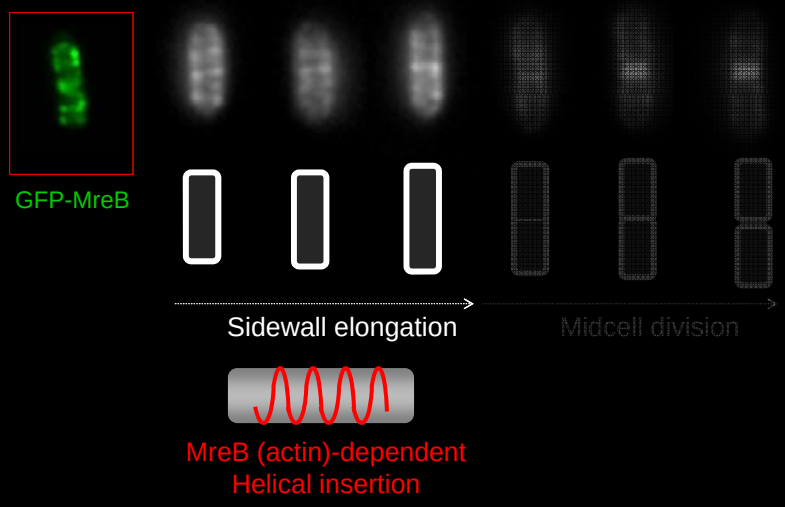
MreBs
(actin cytoskeleton)



Peptidoglycan
cell wall

What is their relationship?

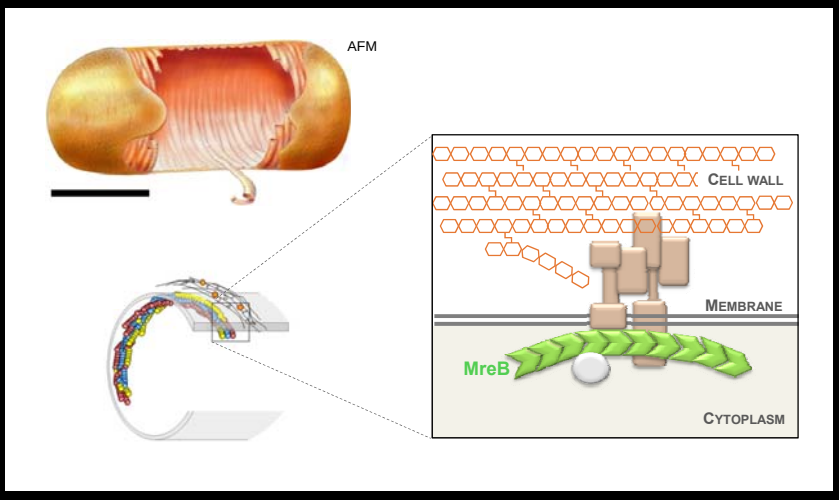
Fluorescent labelling of new cell wall insertion



(Daniel & Errington. ***Cell*** 2003, 113:767-776)

2001-2011, a decade of 'helical' research:

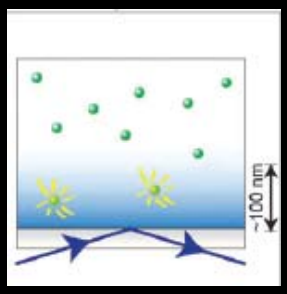
Model for the organization of cell wall synthesis by actin-like MreB proteins



Adapted from Carballido-López *et al.* (2006) ***Dev. Cell*** 4:19-28

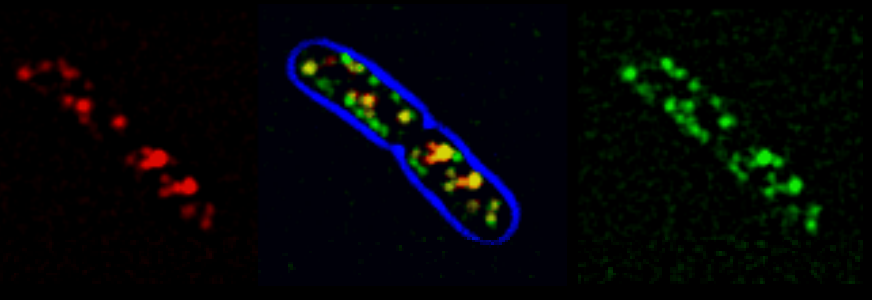
A fresh look to the spatial and temporal organisation of the MreB cytoskeleton by TIRF

Total **I**nternal **R**eflection
Fluorescence Microscopy



MreBs form diffraction-limited motile patches in exponentially growing cells

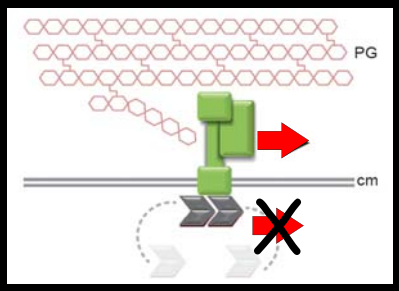
TIRF BF EPI



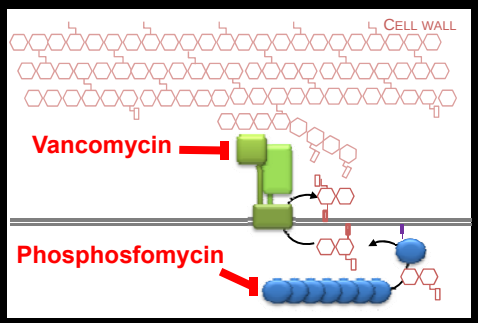
MreBs move:
- ✓ circumferentially
- ✓ bidirectionally
- ✓ processively

3 MAJOR NEW INSIGHTS:

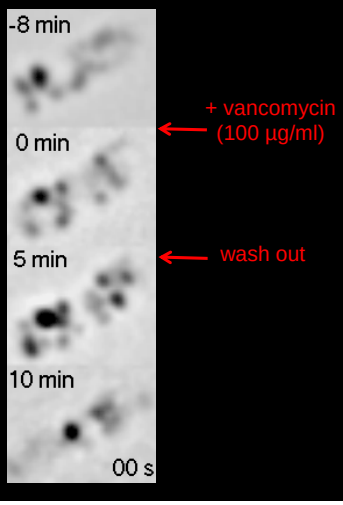
1. MreBs form dynamic patches and not extended helices during exponential growth
2. MreB-associated elongation complexes display processive, circumferential movement
3. Motion of MreB patches is not driven by treadmilling but by PG synthesis itself



Inhibition of PG synthesis stops MreB patches motility



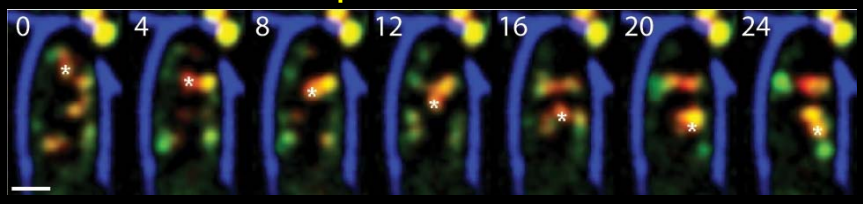
reversible inhibition of MreB movement



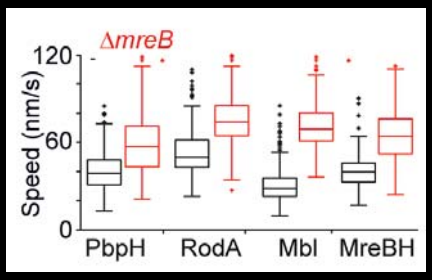
What, then, is the biological function

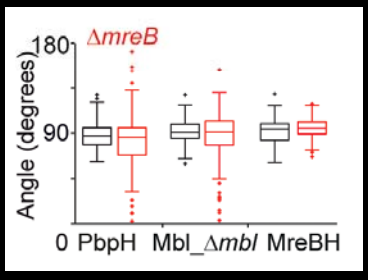
of the essential actin-like MreB?

In the absence of MreB, cell wall synthesis complexes run amok

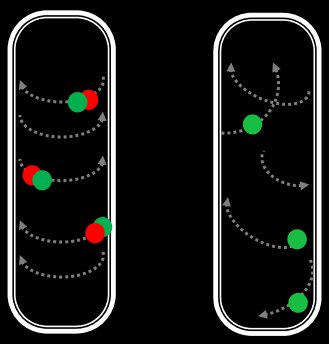


Patches move much faster & exhibit less uniform directionality

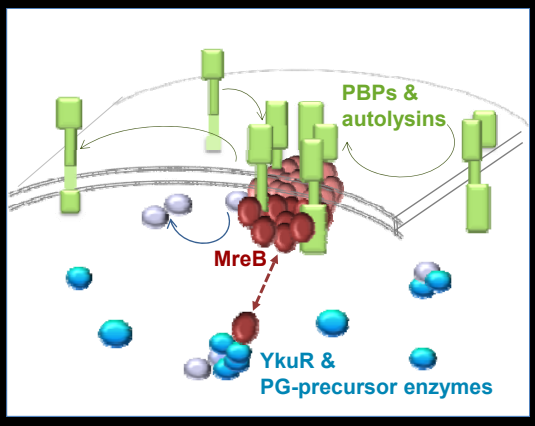




In the absence of MreB, cell wall synthesis complexes run amok

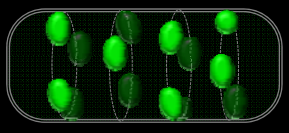


with MreB | without MreB

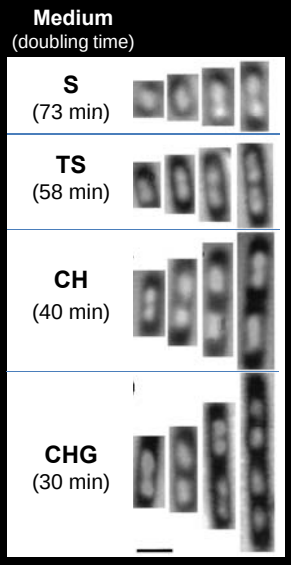


Model for the coordination of extra- and intra-cellular PG-synthesizing machineries by MreB

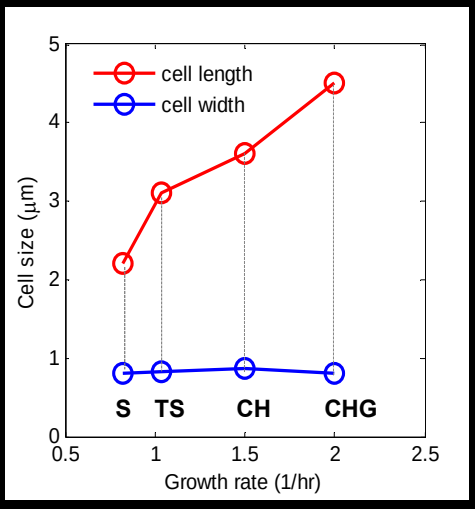
How does circumferential motion of cell wall elongation machineries correlate with cell wall growth?



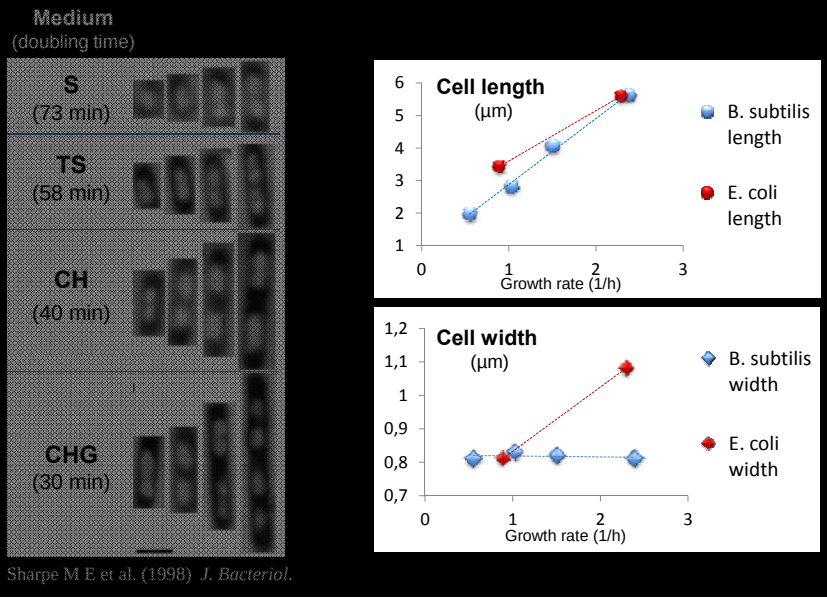
B. subtilis cell length (but not cell width) is regulated in response to nutrient availability



Sharpe M E et al. (1998) *J. Bacteriol.*



E. coli cell length and cell width are regulated in response to nutrient availability

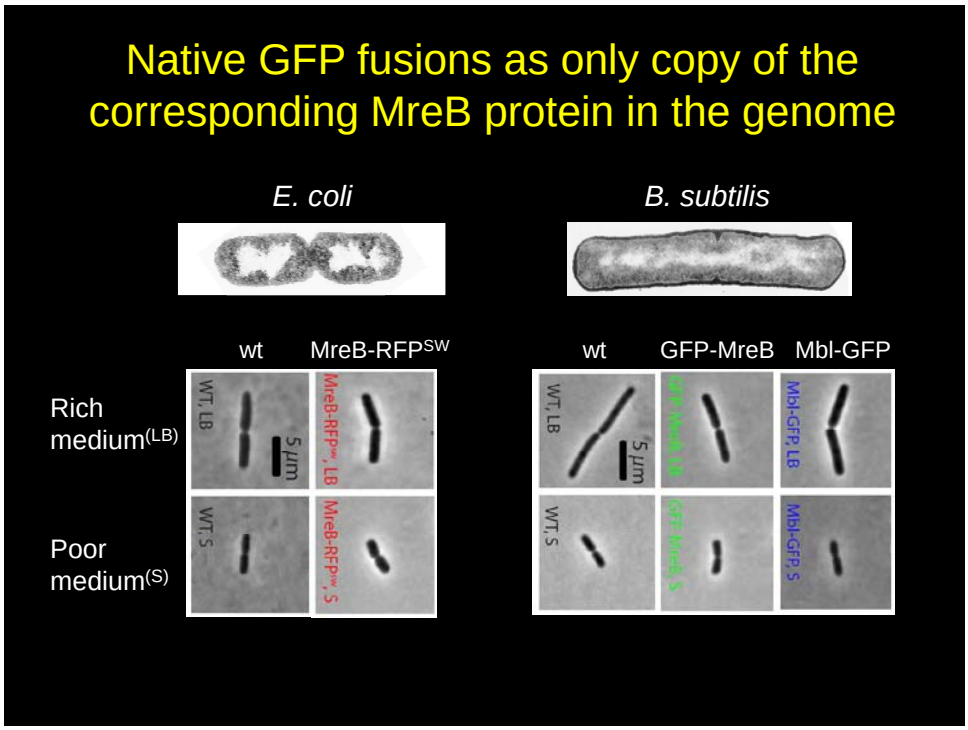


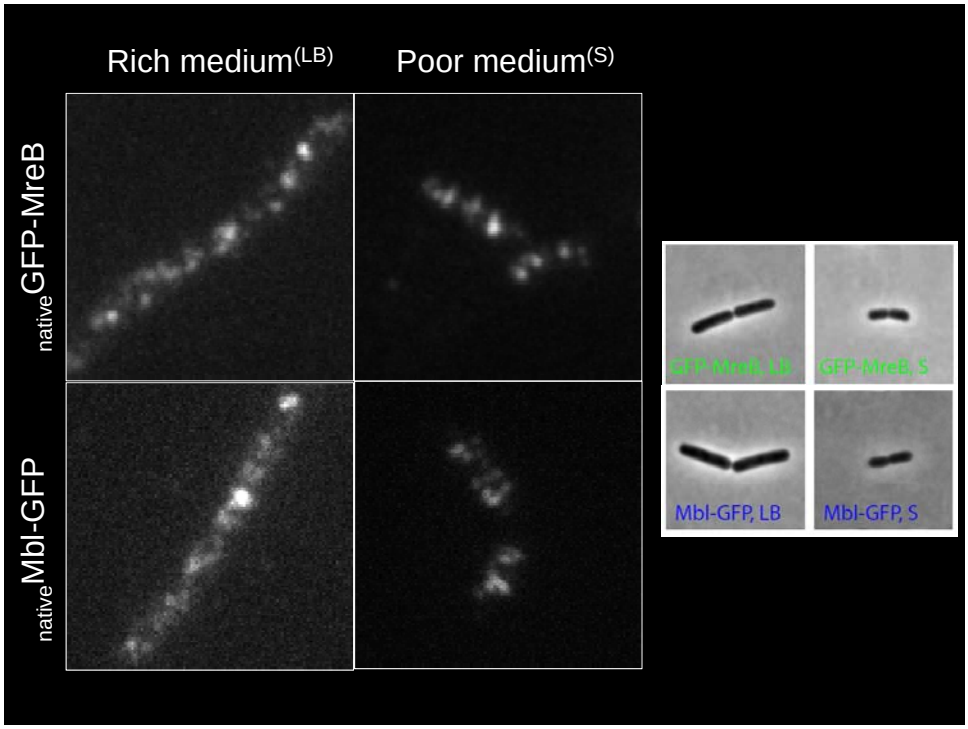
Sharpe M E et al. (1998) *J. Bacteriol.*

In response to nutrient availability,

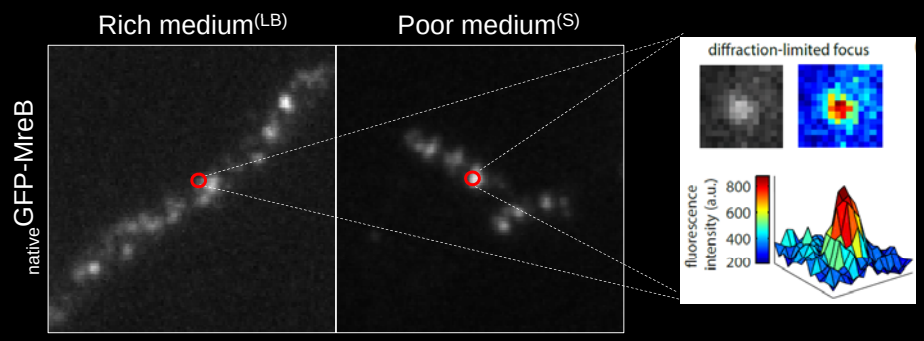
do rod-shaped cells regulate:

1. *rotation speed of MreB patches ?*
2. *density of patches per unit surface area?*
3. *size of the patches/polymers?*

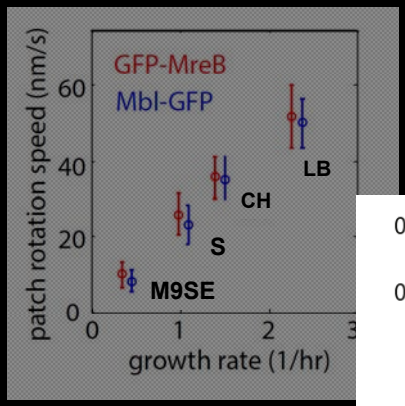

Native GFP fusions as only copy of the corresponding MreB protein in the genome
E. coli
B. subtilis
wt
MreB-RFPSW
wt
GFP-MreB
Mbl-GFP
Rich medium(LB)
Poor medium(S)
WT, LB
MreB-RFPSW, LB
WT, S
MreB-RFPSW, S
GFP-MreB, LB
GFP-MreB, S
Mbl-GFP, LB
Mbl-GFP, S
5 µm


Rich medium(LB)
Poor medium(S)
nativeGFP-MreB
nativeMbl-GFP
GFP-MreB, LB
GFP-MreB, S
Mbl-GFP, LB
Mbl-GFP, S

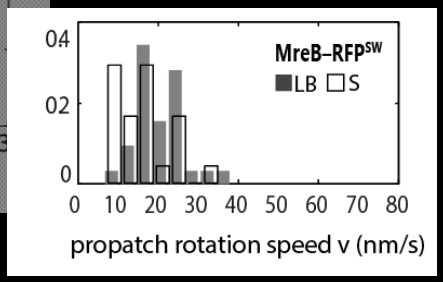
Diffraction-limited patches are the physiological form of MreB in both conditions



MreBs patch speed is proportional to growth rate in *B. subtilis*, but not in *E. coli*



B. subtilis

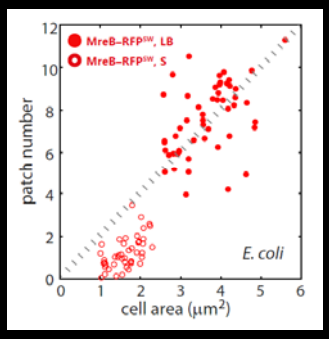


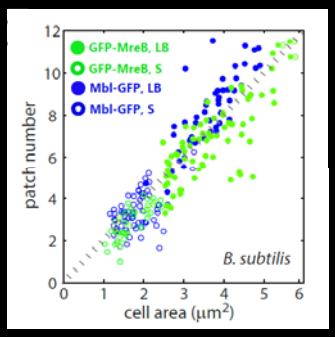
E. coli

MreBs patch density is constant at different growth rates in *B. subtilis*, but not in *E. coli*

E. coli

B. subtilis





TAKE-HOME MESSAGE:

B. subtilis regulates patch rotation speed, not patch density, in response to growth rate

E. coli regulates patch density, not rotation speed, in response to growth rate

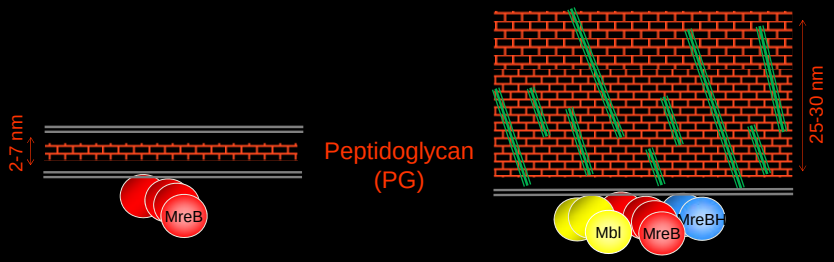
Distinct regulatory mechanisms reflect different cell wall integrity constraints in Gram+ and Gram-

E. coli

B. subtilis

Gram -

Gram +



SUMMARY

- ✓ The actin-like MreB cytoskeleton and the cell wall are major determinants of shape in rod-shaped bacteria
- ✓ MreB proteins form motile patches in growing cells
- ✓ MreB assemblies organize and restrict the motility of cell wall synthesizing machineries in the membrane
- ✓ Movement of MreB-associated cell wall elongation machineries and cell wall expansion are correlated

The MreB cytoskeleton and cell wall synthesis are prominent targets for new antibiotics!