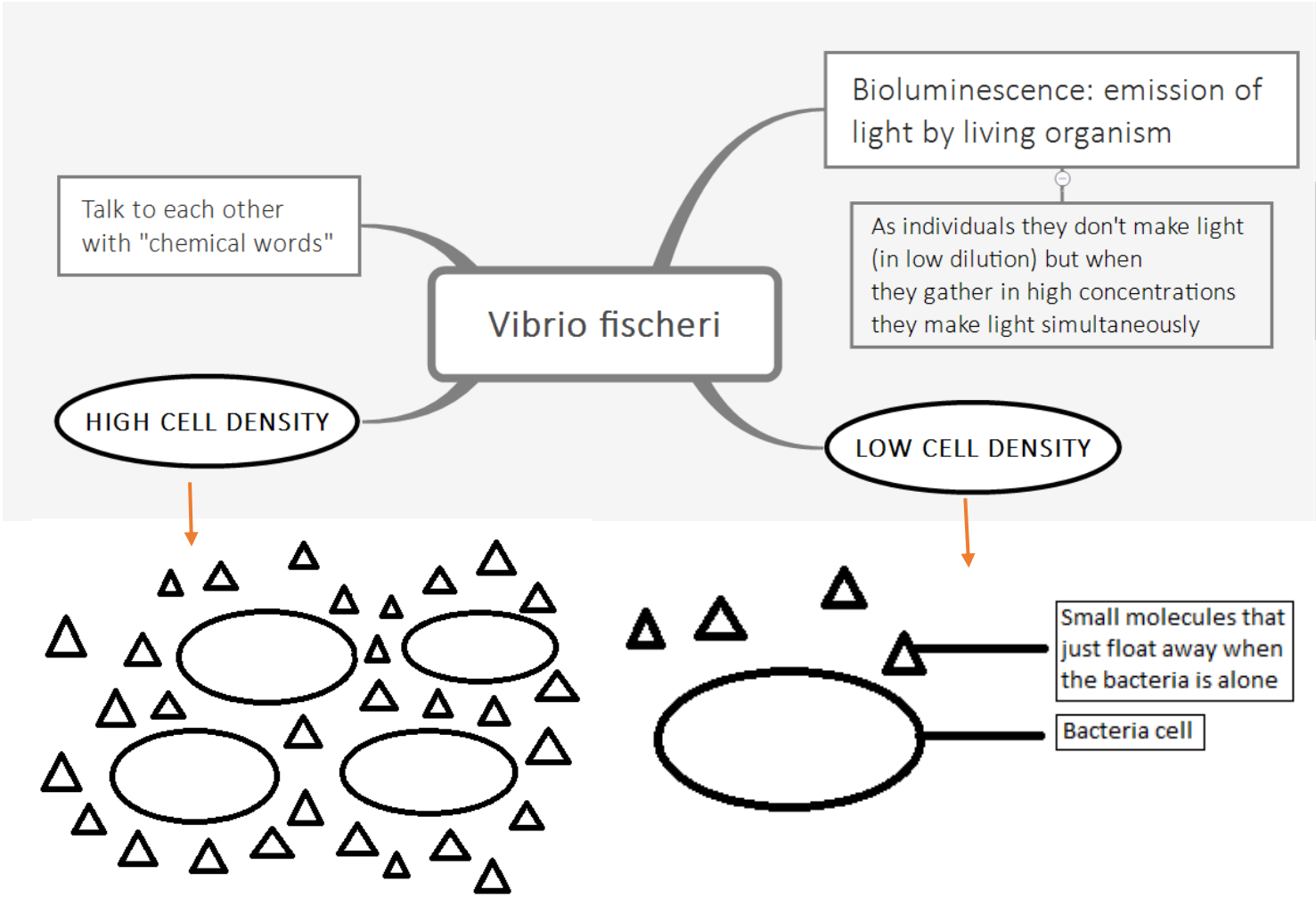
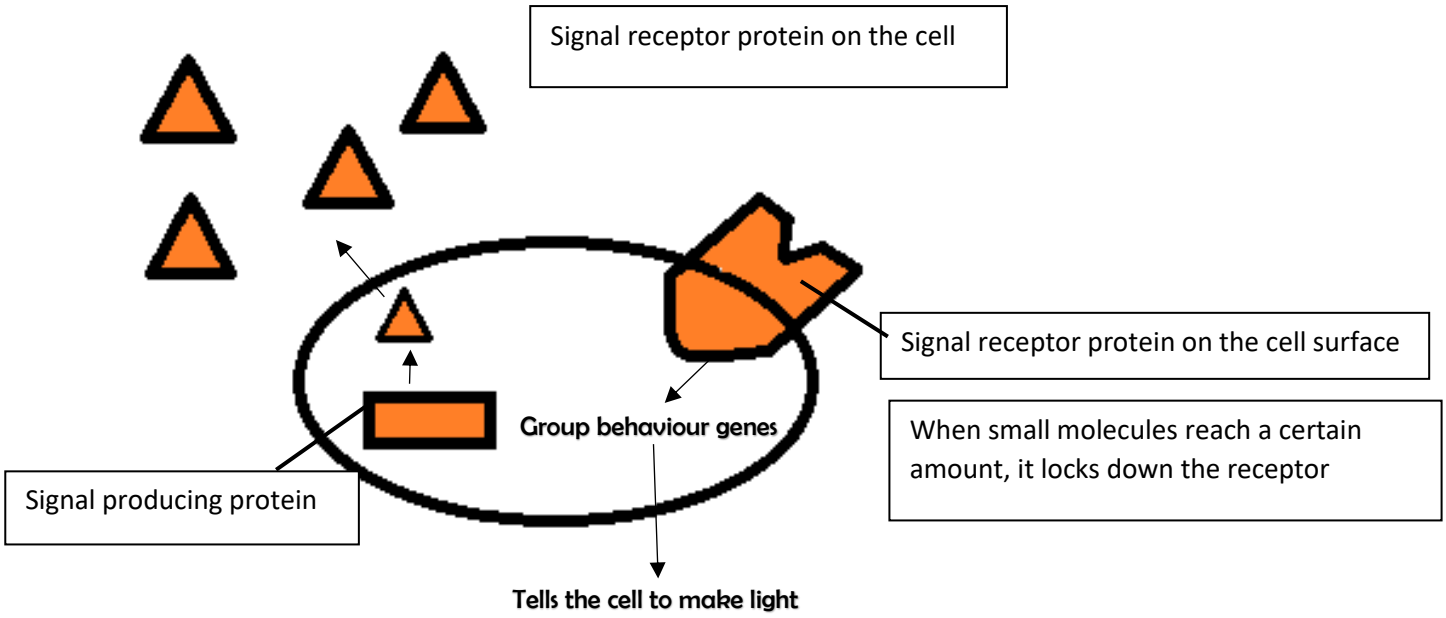
Lecture 11: Quorum Sensing

- Marine bacteria that comes from the ocean: Vibrio fischeri



Bacterial Quorum Sensing

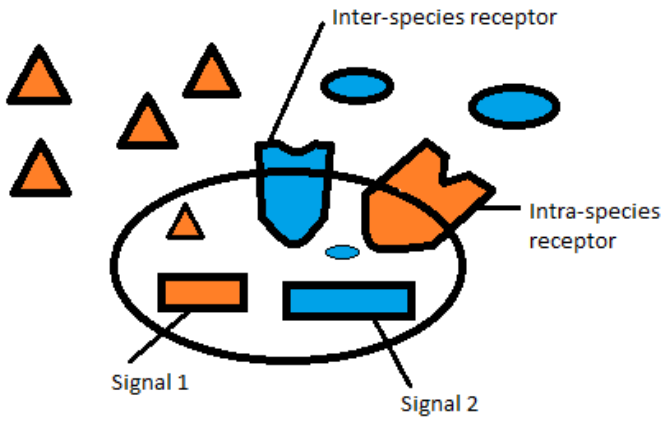
- All bacteria have systems like this, all bacteria can talk to each other
- All the bacteria launch their virulent attack together
- Bacteria always control pathogenicity through quorum sensing



Intra-species communication

- Each bacterium uses a particular molecule that is its own language & allows it to count its own siblings

Multi-lingual Bacteria



- There is a universal communication molecule for all bacteria.
- Bacteria can distinguish themselves from other bacteria

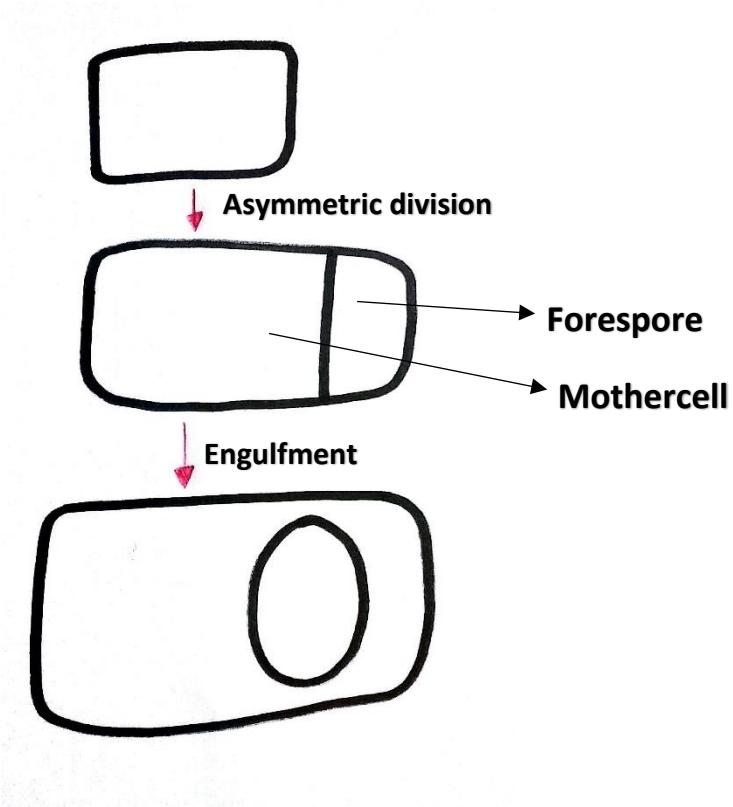
Lecture 13: Spore formation in *Bacillus subtilis*

- Bacillus anthracis (Anthrax) discovered by Robert Koch

How *B. subtilis* makes a spore

- Spore formation is a tale of TWO cells
- Cell-specific transcription factors drive gene expression
- The 2 cells "talk" to each other

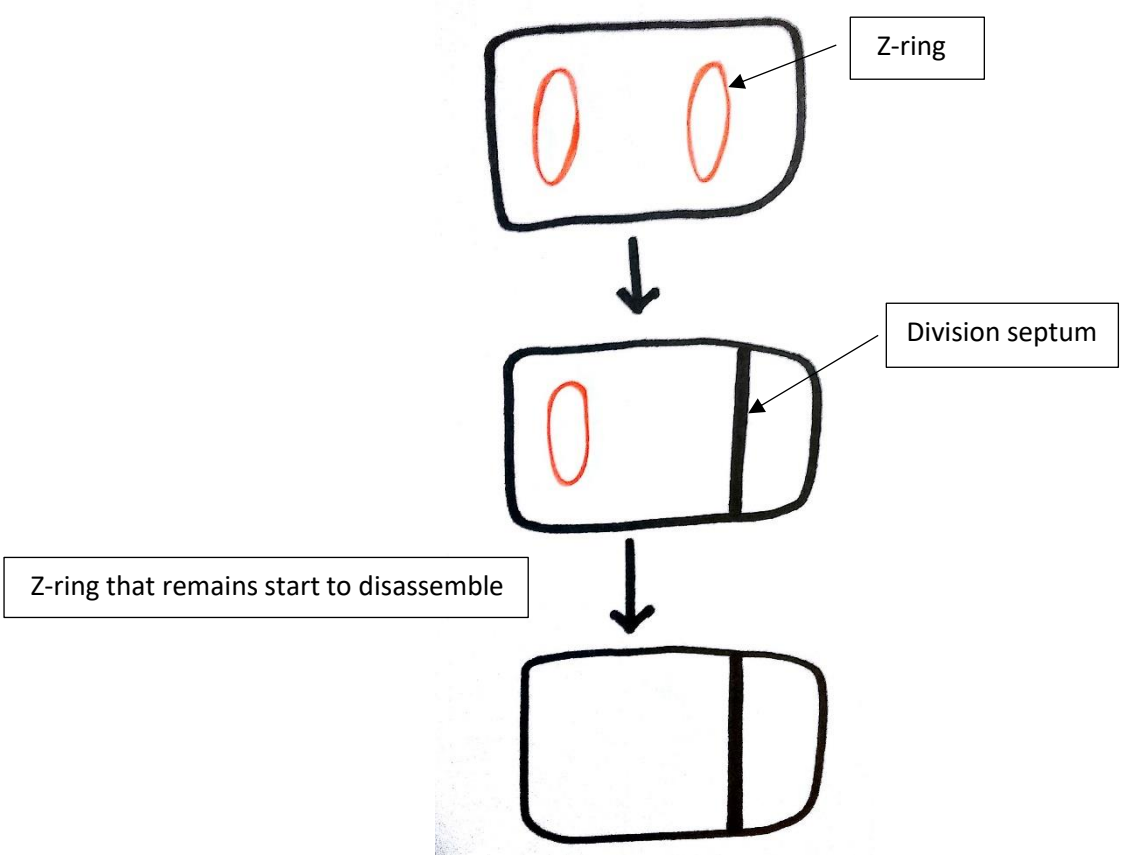
Let's look at the principle stages of spore formation:



- The TWO cells are not completely independent although they each follow their own program of gene expression
- Spore formation is triggered by nutrient limitations and that results in cells entering a pathway to form 2 DIFFERENT cells
- The pre-divisional sporangium has two chromosomes
- The forespore is destined to become the spore, whereby the mother cell nurtures the forespore and eventually liberates the forespore by lysing

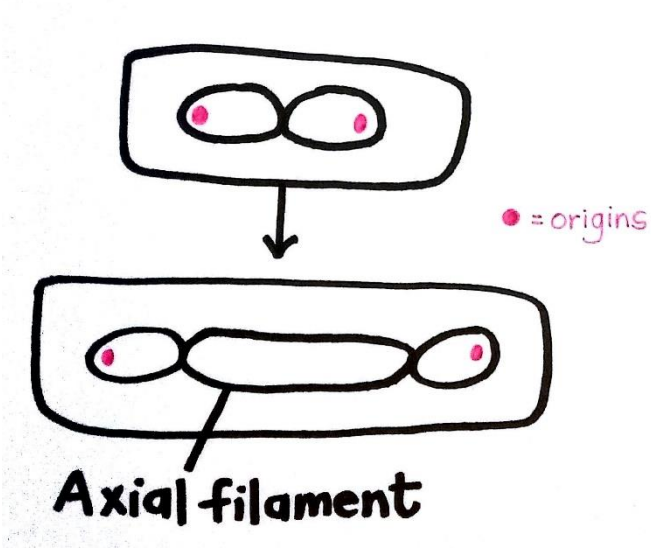
What is the process?

- Septation is governed by the tubulin-like protein **FtsZ**, this forms the Z-ring
- Higher cells rely on actin, bacteria rely on tubulin
- Only one of the Z-rings gets converted to a division septum. The other Z-ring gets disassembled.



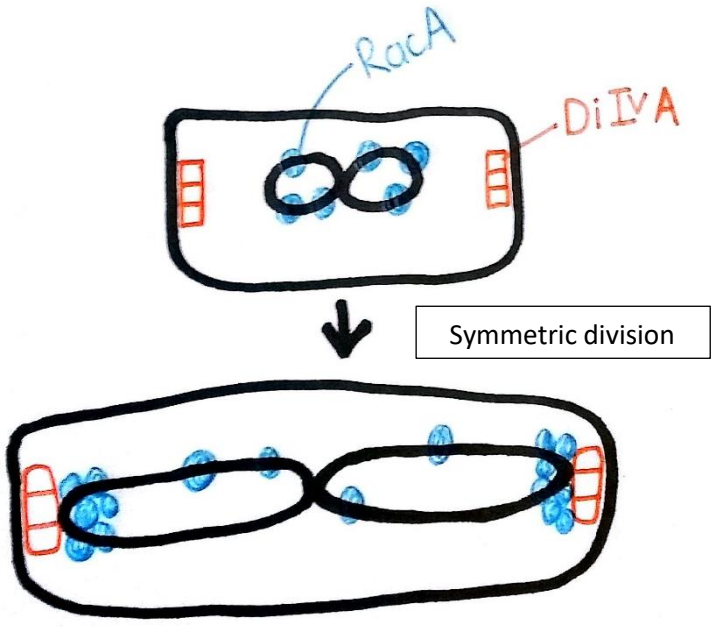
Now, how do bacteria ensure that each new cell gets a chromosome?

- Nucleoids gets remodelled to axial filament which extends across the cell
- Each of the origins located at extreme opposite poles to sporangium



The process of remodelling the chromosome into an axial filament and anchoring it at the poles is mediated by 2 proteins: **DilVA** & **RacA**

- RacA anchors the origins to DilVA proteins at the poles



- "pumping"of chromosome into the forespore is mediated by a molecular machine, a protein known as DNA Translocase
- Translocase is located at the septum and uses energy from ATP to pump the remaining portion of the chromosome into the forespore compartment
- In sporulating cells, cytokinesis takes place before chromosome segregation
- Translocase can be visualized by tagging it with a green fluorescence protein

Conversion of intercell into a spore involves 3 principle genetic processes:

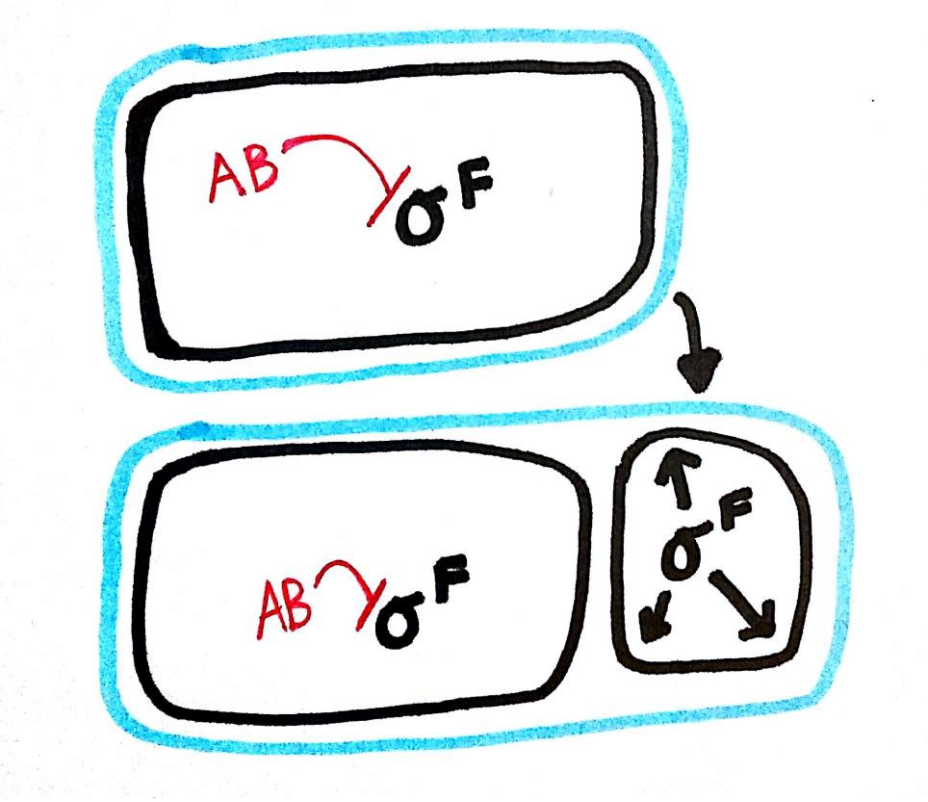
3

1. Remodelling of chromosome into the forespore into the doughnut-like structure in which state it is highly resistant to radiation
2. Formation of thick layer of cell wall material called CORTEX around the forespore
3. Forespore and mothercell have their own pathways of cellular differentiation. Pathway of differentiation are driven by transcription factors that act in a cell-specific manner

FIVE main TF that drives the process of sporulation

- SpoOA
  - Master regulator for sporulation
  - Becomes activated in response to nutrient limitations
- σ^F
  - RNA pol sigma factor
  - Becomes activated in forespore comparment
- σ
- σ^G
  - Replaces
  - Forespore compartment
- σ^K

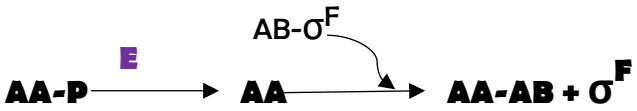
Activation of σ^F



- σ^F is synthesized in the pre-divisional sporangium but not activated there
- AB is an "antisigma-factor" that holds σ^F inactive
- AA is an "anti-antisigma-factor" that frees σ^F from AB

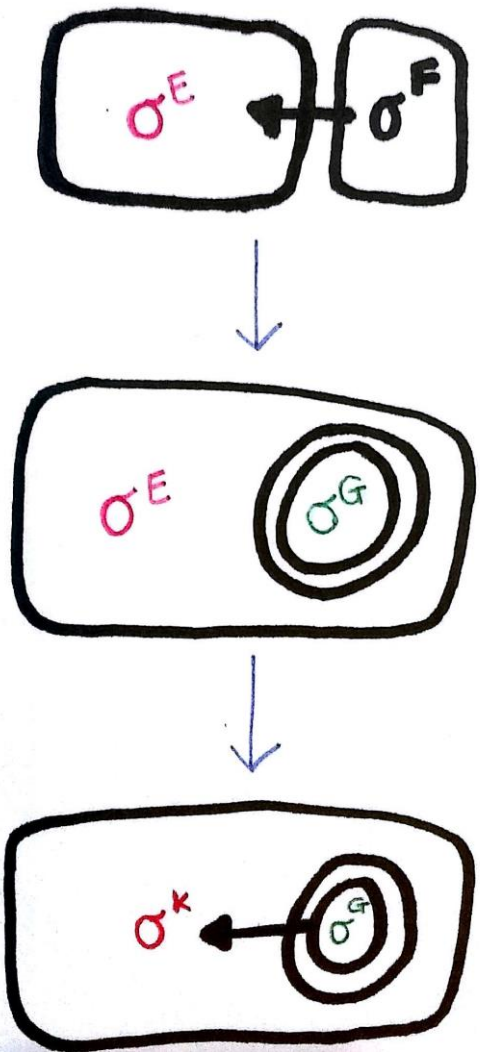
AA + = AA-AB + σ^F

- AA is regulated by phosphorylation. It is a phospho-protein. In its phosphorylated state it is INACTIVE and in its dephosphorylated state it is ACTIVE and capable of triggering the activation of σ^F
- The conversion of phosphor-form to dephospho-form is mediated by a phosphatase called **E**



- E phosphatase localizes to the septum and activates σ^F in the forespore

A two-way conversation



- Sigma F sends a signal across the membranes that separate the TWO cells that leads to the activation of sigma E in the mother cell.
- Sigma E sends a signal that leads to the activation of sigma G in the forespore cell
- Sigma G sends a signal back to the final transcription factor, sigma K, to appear

- Sigma K is initially synthesized as an inactive pro-protein (primary gene product has an N-terminal extension of approximately 20 amino acids that renders pro-sigma K inactive

How does it become ACTIVE?

- PROTEASE needs to "chop off" extension to generate maturing active form of the transcription factor
- Sigma K maturation depends on Sigma G
- Activation of sigma K in one cell depends on genetic events of adjacent cell
- Inhibitory proteins hold protease inactive

- Sigma G TURNS ON the gene encoding the signalling protein and that signalling protein is secreted across the membrane of the forespore where it interacts with a complex of proteins that includes the protease and its inhibitory proteins and reverses the inhibition so that cleavage can occur

Protease

- Bacterial protease has homology to mammalian protease that activates transcription factor for cholesterol metabolism
- Active site is located on the membrane
- N-terminal extension of sigma K inserts into a cavity into the membrane by protease