# Mathematical modeling of a two-component system (PhoQ/PhoP Signaling System) in *Escherichia coli*

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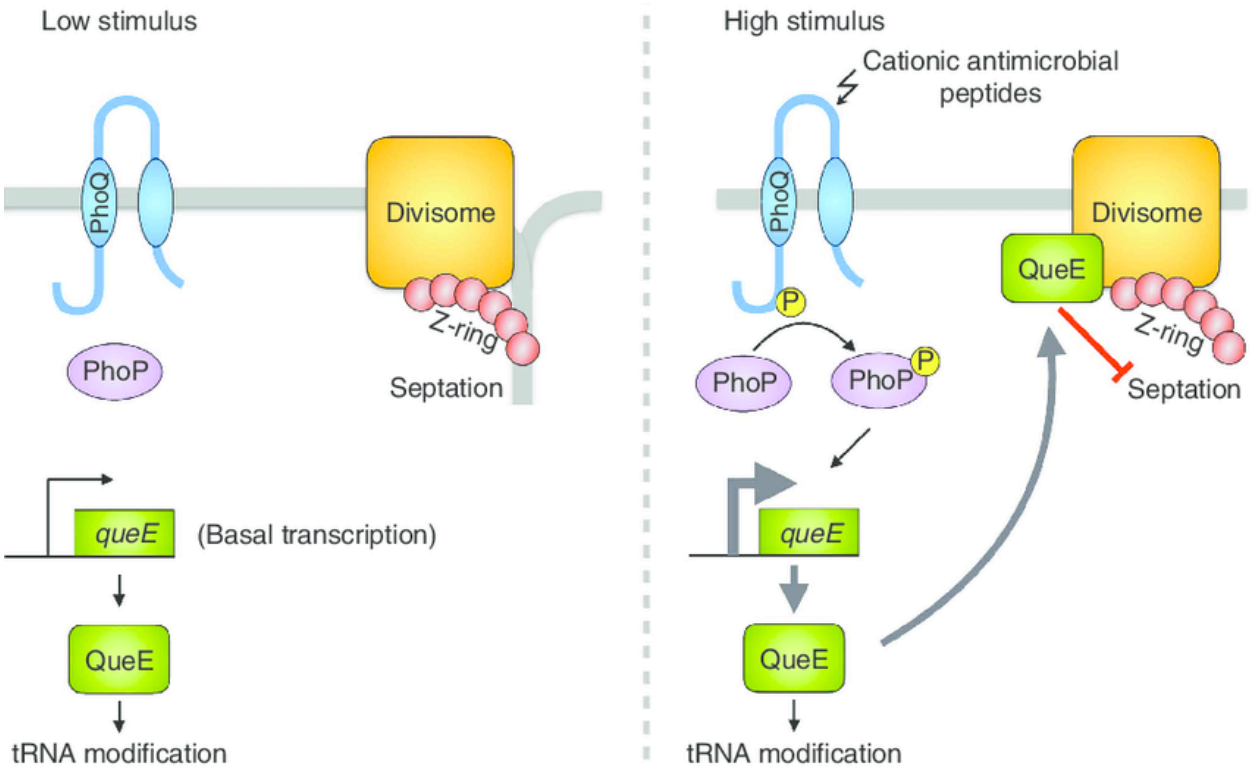
## PhoQ/PhoP network in *E.coli*




Source:
https://www.researchgate.net/publication/305726975/figure/fig2/AS:389787761561601@1469944052431/E-coli-PhoQ-PhoP-two-component-system-modulates-cell-division-via-QueE-PhoQ.png

**Activation of PhoQ**: The presence of CAMPs is detected by the sensor kinase PhoQ. This detection leads to the **autophosphorylation of PhoQ**, where it adds a phosphate group to itself. This phosphorylation is a critical step in the signaling cascade, as it activates PhoQ's function.

**Phosphorylation of PhoP**: Once PhoQ is autophosphorylated, it **transfers the phosphate group to the response regulator PhoP**. This phosphorylation activates PhoP, allowing it to function as a

**transcription factor**.

**Activation of the queE Gene**: Activated **PhoP binds to the promoter region of the queE gene**, leading to its transcription. The **queE gene encodes the enzyme QueE**, which is involved in the **modification of tRNA through the biosynthesis of queuosine**, a hyper-modified guanosine found in certain tRNAs.

**Production of QueE Protein**: The transcription of queE results in the translation of the QueE protein. This **protein plays a dual role: it is involved in tRNA modification and also has a significant impact on cell division**.

**Binding to the Divisome**: Under conditions of stress, such as the presence of CAMPs, **QueE localizes to the divisome**, which is the cellular machinery responsible for cell division. The divisome includes proteins like FtsZ that are essential for forming the division septum.

**Inhibition of Septation**: **By binding to the divisome, QueE interferes with the normal process of septation**, which is the formation of the septum that separates the two daughter cells during division. This interaction leads to filamentation, where the cells elongate but do not divide properly, resulting in long, filamentous cells.

## Flatfile

| Source | Target | Edge Type | Interaction Type | Experimental Evidence | Reference (PMID) |
|---|---|---|---|---|---|
| cationic antimicrobial peptides | PhoQ | Activation | Autophosphorylation | **The measured phosphorylation state** of PhoQ in the presence of peptides (likely using phosphorylation-specific assays). | https://www.researchgate.net/figure/E-coli-PhoQ-PhoP-two-component-system-modulates-cell-division-via-QueE-PhoQ_fig2_305726975 |
| PhoQ | phoP | Activation | Phosphorylation | Detected phosphorylated PhoP (PhoP-P) using techniques like Western blotting. | |
| PhoP | queE | Activation | Transcription activation | Reporter assays or quantitative PCR showed increased queE transcription when PhoP | |

| | | | | | |
|---|---|---|---|---|---|
| | | | | was activated. | |
| queE | QueE | Activation | Translation | Demonstrated by observing increased QueE protein levels, likely through protein quantification assays like Western blotting. | |
| QueE | tRNA modification | Activation | Functional modification | Indirectly inferred from downstream effects on tRNA modification, supported by the role of QueE in modifying tRNA in similar contexts. | |
| QueE | Septation | Inhibition | Associates with the divisome | **Filamentation Observation**: Imaging experiments using a YFP-QueE fusion protein showed QueE localization at the divisome in filamentous cells, supporting its role in inhibiting division. | |

# The above network is unique to E coli - QueE activation

**Discussion**

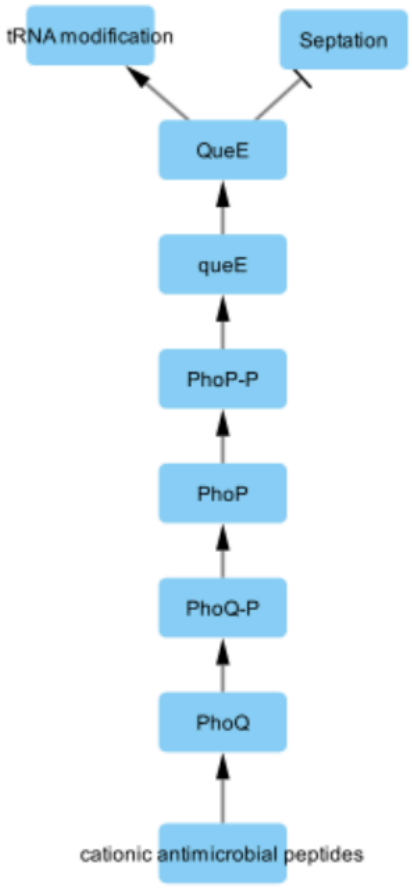
The filamentation described here is not a result of antimicrobial peptide-induced envelope damage or other toxic effects of the peptides. Rather, the septation block is caused by high-level activation of the PhoQ/PhoP signalling system that is induced by the antimicrobial peptides[6] (Fig. 6). We find that growth in medium with low levels of divalent cations, another condition that stimulates PhoQ[4], produces similarly high levels of PhoQ activation in strains that lack the negative regulator MgrB, and also leads to filamentation. By isolating and analysing suppressor mutations, we determined that the cell division block is caused by an increase in transcription of *queE*, a gene encoding an enzyme in the queuosine tRNA modification pathway. QueE along with other enzymes required for the initial steps of queuosine biosynthesis (QueC, QueD, QueF) were first identified in *Bacillus subtilis* by comparative genomics combined with experimental validation in *Acinetobacter baylyi*[31]. *B. subtilis* QueE was subsequently studied in detail and shown to be a CDG synthase[22,23,32,33] (Supplementary Fig. 8). Homologues of QueE, QueD and QueC are present in *E. coli* and their role in queuosine biosynthesis has been confirmed[42–44]. However, we find that QueE-induced filamentation does not depend on QueD and QueC. Thus, it appears that *E. coli* QueE has another role in addition to queuosine biosynthesis. It is also noteworthy that *E. coli* QueE shares only 20% identity at the amino-acid sequence level with the *B. subtilis* QueE (and shares only 40% similarity—based on a T-Coffee alignment[45]). In addition, the genes *queC*, *queD*, *queE* are not co-transcribed in *E. coli*, and are in fact somewhat dispersed around the *E. coli* genome, in contrast with their organization in a single operon in *B. subtilis*. These observations suggest a significantly different evolutionary history for *E. coli* QueE compared with its better-studied homologue from *B. subtilis*, consistent with an additional function for the *E. coli* protein.

The localization of YFP-QueE to the divisome in filamenting cells, and the absence of this localization in cells for which filamentation has been suppressed by mutations in *ftsA* or *ftsZ*, suggests that filament formation depends on a direct interaction between QueE and divisome components. The YFP-QueE localization in cell filaments is always observed on top of considerable diffuse cytoplasmic YFP-QueE fluorescence. This observation is consistent with the fact that filamentation emerges only when *queE* expression is increased above the significant basal expression level that is independent of PhoQ/PhoP (Fig. 4d), and suggests QueE binds the divisome with low affinity. Hence, only under conditions of strong PhoQ/PhoP stimulation are QueE levels high enough to bind the divisome and inhibit septation.

In filamenting conditions, we find that the distributions of cell/filament lengths are highly heterogeneous, with the average length increasing with QueE expression. Furthermore, for less-severe filamentation, many small cells are also produced (Supplementary Table 1). We note that septation must occur at a low but non-zero frequency in filamenting cells since otherwise the cultures would consist of only a small number of extremely long cells. The wide distribution of cell lengths and the appearance of small cells suggest a model in which the locations of infrequent septation events are randomly distributed along the length of the cell filaments. Small cells will be produced when septation occurs near the filament ends; the proportion of such events will be higher when the average filament length is shorter. Taken together, these observations suggest that PhoQ/PhoP tunes the frequency of septation by adjusting *queE* expression in response to the level of stimulus.

A variety of stresses cause *E. coli* and related bacteria to grow as filaments. Examples include DNA damage, exposure to antimicrobial compounds, high hydrostatic pressure, and osmotic stress (reviewed in[46]). In some cases, the block in septation is caused by a stressor directly inhibiting a component of the cell division machinery. For example, some beta-lactam antibiotics inhibit penicillin-binding protein 3 (FtsI)[47]. In other cases, the septation block is caused by a regulatory circuit that has detected the stressor or associated environmental condition[48]. This control of division by the cell is generally assumed to increase fitness in the presence of the stress. However, the selective advantage is not always easy to discern. One well-studied example is the *E. coli* SOS response, in which detection of DNA damage leads to upregulation of the cell-division inhibitor SulA[26,27]. SulA-dependent filamentation is often described as a division checkpoint that protects cells from the potentially lethal

# Cytoscape network




| Source | Target | Edge Type |
|---|---|---|
| cationic antimicrobial peptides | PhoQ | + |
| PhoQ | PhoQ-P | + |
| PhoQ-P | PhoP | + |
| PhoP | PhoP-P | + |
| PhoP-P | queE | + |
| queE | QueE | + |
| QueE | tRNA modification | + |
| QueE | Septation | - |