**The MksG nuclease is the executing part of the bacterial plasmid defense system MksBEFG**

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**Presenter:** Kamryn Escalante **Date/ Time:** 2024/03/28, 15:10- 16:00

**Commentor:** Professor Pei-Jane Tsai **Location:** Room 601, Med College Building

**Background:** Horizontal gene transfer is the primary mechanism of genetic exchange and recombination in prokaryotic organisms. As a result, cells constantly face the threat of acquiring foreign DNA which can potentially harm its genomic integrity. Overtime, bacteria have evolved various strategies to defend against invading DNA molecules. Gram-positive bacterium *Corynebacterium glutamicum* consists of a MksBEFG complex that shares homology with the MukBEF condensing system found in Gram-negative bacteria. However, the function of MksBEFG in *C. glutamicum* remains unknown.

**Objective/ Hypothesis:** To investigate the molecular organization of the MksBEFG complex in *C. glutamicum* and its potential role in providing enzymatic activity for defense against plasmid.

**Results:** The MksBEFG complex in *C. glutamicum* plays a role in controlling plasmid copy number. Isolating of transformed plasmid DNA from various strain backgrounds showed that deleting *mksB*, *mksF* or *mksG* led to increased plasmid levels, as measured by qPCR. This indicates that these three major subunits of the Mks system, along with MksG, are crucial for effective plasmid defense. Furthermore, an ATP hydrolysis assay demonstrated that MksF and MksE stimulate the ATPase activity of MksB. Additionally, MksB alone can bind and shift DNA in gel electrophoretic mobility shift assay. To further discover the assembly of the MksBEFG complex, Bio-layer Interferometry was used to examine the interaction between the subunits. Based on the results, MksF acts as an interaction hub between MksBEF complex and MksG subunit. The primary focus in controlling foreign DNA lies with a nuclease, as demonstrated by their findings that MksG actively degrades plasmid DNA. Single-molecule localization microscopy suggests that the dynamics of MksG are influenced by MksB and by the presence of plasmid DNA.

**Conclusion:** The nuclease activity of MksG plays a vital role in the MksBEFG system's defense against foreign plasmid DNA entering *C. glutamicum*.