**INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI**
**SHORT ABSTRACT OF THESIS**

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Thesis Title: Molecular investigations of cellular roles of calmodulin and calcium/calmodulin-dependent kinases in stress responses, asexual and sexual developments in *Neurospora crassa*

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## SHORT ABSTRACT


Calcium ($Ca^{2+}$) plays a primary role in regulating numerous cellular processes and adaptive responses in eukaryotes. The binding of $Ca^{2+}$ changes the conformation and charge of the protein, which are the basis for signal transduction. Calmodulin (CaM) is the principal $Ca^{2+}$ binding protein expressed in all eukaryotes including *Neurospora crassa*. When bound to $Ca^{2+}$, CaM activates over 300 target proteins. $Ca^{2+}$/CaM-dependent kinases ($Ca^{2+}$/CaMKs) are one of the main effector proteins of CaM. Preliminary studies in *N. crassa* have shown the importance of CaM and $Ca^{2+}$/CaMKs in growth, development, stress response, sexual development, and in regulating the circadian system. However, the detailed cellular roles and molecular mechanism by which CaM and $Ca^{2+}$/CaMKs regulate the different phases of life cycle or the cell under different conditions still remain unclear in *N. crassa*. Therefore, this study sought to understand the cellular roles and the mechanism by which CaM and $Ca^{2+}$/CaMKs mediated the cell under stress conditions, during sexual development, and in regulating the circadian clock.

To study the cellular roles of calmodulin (CaM) and calcium/ calmodulin-dependent kinases ($Ca^{2+}$/CaMKs), we used a CaM mutant strain *cmd*$^{RIP}$ (#26), generated using repeat-induced point (RIP) mutation and the knockout mutant strains of four $Ca^{2+}$/CaM Kinases ($Ca^{2+}$/CaMKs) Δ*camk-1*, Δ*camk-2*, Δ*camk-3*, and Δ*camk-4*. The *cmd*$^{RIP}$, Δ*camk-1*, and Δ*camk-2* mutants showed defects in growth, and reduced survival rates under temperature, pH, oxidative, and ER stress conditions. In sexual development, the *cmd*$^{RIP}$ mutant was unable to produce protoperithecium and showed to be female sterile. In addition, the

*cmd*$^{RIP}$ mutant strain was unable to support the wild type perithecia graft, showed defects during cell fusion, and a drastic decline in life span. Furthermore, the circadian-regulated conidiation studies showed that the Δ*camk-1*, Δ*camk-2*, Δ*camk-3*, and Δ*camk-4* mutants increase in the circadian period length without affecting the temperature compensation.

To understand the mechanism of the CaM and $Ca^{2+}$/CaMKs during stress and sexual development, real-time gene expression analysis was performed which showed that the *cmd, camk-1*, and *camk-2* genes were upregulated in the wild type under heat stress and during nitrogen-starved conditions. In addition, the relative expression of genes encoding the heat shock proteins *hsp70* and *hsp80*, and pheromone response pathway genes *pre-1*, *pre-2*, *ccg-4*, *mfa-1*, and *fmf-1* were reduced in the *cmd*$^{RIP}$, Δ*camk-1*, and Δ*camk-2* mutant strains which suggested that CaM and CaMKs are involved in regulating the heat shock response and pheromone response pathways in *N. crassa*.