



Centre for BioSystems Science and Engineering

THESIS COLLOQUIUM

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MRDG Seminar Hall
Biological Science Building
Indian Institute of Science

Insights into signalling and crosstalk in Two-component signalling systems of *Mycobacterium tuberculosis*

Mr. Gaurav D. Sankhe

Advisers: Prof. Deepak K. Saini and Prof. Narendra M. Dixit.

Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), is one of the leading causes of deaths in humans. Mtb, like other prokaryotes, senses and responds to changes in its environment primarily through two-component signalling systems (TCSs). A TCS consists typically of a trans-membrane sensor histidine kinase (HK) and a cytosolic response regulator (RR). HK detects extracellular cues, following which it gets autophosphorylated and subsequently transfers the phosphoryl group to RR. This phosphorylation of RR not only triggers upregulation of the HK and RR involved through autoregulation but also elicits a transcriptional response essential for adaptation to the cues. Although specificity, where phosphotransfer is restricted from an HK to the RR of a TCS, is the norm, Mtb is an exception displaying extensive crosstalk in vitro, where HKs transfer signals to RRs of other TCSs. This extensive crosstalk in vitro raises many questions. First, what is the extent of this crosstalk relative to the cognate, or specific response? Second, is this crosstalk realized in vivo? Third, what evolutionary advantages are associated with crosstalk given that specificity is the norm? My study addresses some of these questions using a mix of biochemical, mathematical and in vivo approaches.

First using the previously identified in vitro crosstalk map, I aimed to identify the pathways that are kinetically feasible. For this, I optimized a high throughput assay and established its equivalence to classical PAGE/ autoradiography. Historically, autophosphorylation of HKs was thought to occur in trans. Using wild-type and mutant proteins, I performed dimerization assays, thermophoretic affinity measurements, and competition-based phosphorylation assays and established that the autophosphorylation of the HK MtrB occurs in cis, as does that of the HKs PhoR and PrrB. A mathematical model we developed based on this cis mechanism captured experimental data. The fits yielded estimates of the autophosphorylation rates. Next, I probed the ability of the autophosphorylated HKs to transfer phosphoryl groups to their cognate and non-cognate RR partners. The first step here is binding with these partners. I evaluated the binding affinities using Micro Scale Thermophoresis (MST). Remarkably, I found that every HK bound a non-cognate RR with higher affinity than its cognate partner. This suggests that phosphorylated HKs could be sequestered by noncognate RRs. This sequestration could be a general mechanism of preventing response via the cognate pathways to short-lived cues. Lastly, to probe crosstalk in vivo, I engineered and utilized a promoter reporter assay. The assay showed that crosstalk indeed occurs in vivo. Taken together, I have developed measurements and analysis tools for quantifying crosstalk in TCS systems, demonstrated crosstalk in vivo, and identified a possible evolutionary benefit, in terms of buffering the systems from noise via sequestration, that such crosstalk can confer on Mtb.

For more information depicting the research

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