Dissecting the role of polynucleotide phosphorylase in virulence gene expression in *Salmonella enterica*

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Almost all forms of life (bacteria, plant or animal) have the gene *pnp* that makes the enzyme polynucleotide phosphorylase (in short PNPase). This enzyme is mainly responsible for bacterial mRNA degradation. But different reports suggested that PNPase is also involved in bacterial growth at low temperature and disease production (virulence). Bacterial PNPase structure analysis has revealed that it is a doughnut shaped protein with five distinct regions (domains) which are strongly conserved in different species of bacteria. Among them, the S1 domain is mainly known as an RNA binding domain. The expression of PNPase was found to be controlled by itself. And it was also found that the S1 domain is required for this selfcontrol. A report on *Yersinia*, a bacterium causing mainly food-borne diseases showed that the S1 domain alone could cause similar effects as the whole PNPase on a virulence factor known as type three secretion system (T3SS).

Salmonella enterica (*S. enterica*) is a classic example of a food borne pathogen causing severe infections in a variety of hosts (including human) that can even persist for long time. Its PNPase has been shown to affect the virulence genes during infection of macrophage cells inside human. On the basis of cell surface structures, *S. enterica* has numerous subtypes known as serovars. The serovar Typhimurium (*S.* Typhimurium) responsible for infection in mouse resembles the infection in human caused by the serovar Typhi (*S.* Typhi). So, *S.* Typhimurium is a widely used experimental model to study salmonella infection. Like *Yersinia*, *Salmonella* also requires the T3SS for disease production. The impact of the S1 domain of PNPase on its own expression and on *Yersinia* T3SS motivated to study the influence of PNPase- S1 domain on *Salmonella*'s virulence.

I have been able to express the recombinant S1 domain from plasmid(s) I constructed and purify it. A gene regulation assay (to study effect of gene/protein on the transcription of another gene) was also carried out to examine how PNPase and the S1 domain protein affected the expression of *S.* Typhimurium plasmid virulence (*spv*) genes. This experiment showed that in the infection-simulating environment, the S1 domain of *S. Typhimurium* PNPase itself indeed had profound effect on the expression of the *spv* genes. Finally my findings suggested that in *pnp*-deficient *S.* Typhimurium, the S1 domain protein itself can control the *spvA* expression level negatively almost as effectively as the whole PNPase.

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