Identification of the Phosphorylation Sites on RitR

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Abstract: While iron is essential to life, it can also easily cause toxicity if consumed or absorbed in excess. Every organism has to have a way to control iron uptake, to inhibit excess levels of iron within the cell. In *Streptococcal pneumonia* the iron uptake mechanism is activated by extracellular iron, however the sensory mechanism used to inhibit this uptake is not yet well understood. When iron is sensed extracellularly, a complex known as Stk-P is activated, and in the presence of ATP this molecule will phosphorylate RitR. When RitR is not phosphorylated it is bound tightly to the DNA of S. pneumonia in close proximity to the piu (pneumococcal iron uptake operon), preventing transcription of that portion of the DNA. However, when phosphorylation of RitR occurs, it is no longer bound to the DNA, which then allows transcription to occur. This research focuses on the location of phosphorylation on RitR and how these changes affect the structure and function. This helped to understand how this protein functions and how it interacts with the DNA by analyzing the changes in binding affinity as the protein was modified via phosphorylation. Once the protein was successfully modified the amino acid sequence was determined, and three main phosphorylation sites were located in relatively close proximity of one another. Knowing the location of these sites enabled research to continue by altering the amino acid sequence so that phosphorylation no longer occurred. This would allow further analysis on which sites of phosphorylation are most crucial to the function of this mechanism and where they are located relative to the piu, allowing further understanding of how RitR is involved in this sensory mechanism. Research will continue in further determining how this sensory mechanism works by modifying the protein structure and amino acid sequence, and analyzing the protein function following these alterations.