Abstract

Bacteria are extremely abundant and found in virtually all possible environments. Most environments fluctuate and cellular adaption is therefore crucial for fitness. Adaptability is achieved through regulatory mechanisms, which to a large extend operate at the level of gene expression. Structural and virulence associated traits are usually heavily regulated to ensure optimal fitness as these features often dictates the future outcome of the bacterium. Gene expression is a major point of regulation as transcription and translation are both costly. Regulation at these points prevent build-up of unnecessary products and wasteful use of limited resources. The genetic flow of information is a complex process with a multitude of sub-steps required for successful expression of genes. Each step in the process is a point of regulation, and all steps are exploited to fine-tune expression in response to a myriad of environmental and internal cues. Major mechanisms of transcriptional and translational regulation, often termed post-transcriptional regulation is reviewed in the Introduction section. Until relatively recently, regulation of gene expression was generally believed to occur mainly at the transcriptional level. Studies of gene regulation therefore largely focused on that aspect. As a result, mechanisms of transcriptional regulation are today understood in detail. In recent years however, the role of post-transcriptional regulation has become apparent, though many of the mechanistic details are still not understood. Overall regulatory circuits are another area that is not well described. Though Escherichia coli is the best studied organism, only half of its genes have been studied from a regulatory point of view. To understand how bacteria adapt, we need to understand how bacteria regulate gene expression; both from an overall and a detailed mechanistic point of view. With knowledge on bacterial adaption we will be able to develop novel treatment methods to combat virulent bacteria. This is becoming increasingly important as antimicrobial resistance is rapidly emerging.

In manuscript I we explore the mechanism of a curious case of post-transcriptional regulation of an important structural component in curli biofilms. The mechanism involves inhibition through base-pairing of sRNAs. They bind distal sites of a long 5'-untranslated region of the mRNA encoding the curli master regulator. Usually, sRNAs inhibit translation by base-paring near or over ribosomal binding sites, which subsequently cause degradation of untranslated mRNAs. We demonstrate that binding of sRNAs at the distal sites directly induce cleavage at a nearby A/U-rich region near a stemloop structure of the mRNA, and that this cleavage is the primary cause of translational inhibition. Furthermore, we demonstrate that the A/U-rich region is an Hfq binding site and that cleavage is RNase E dependent. Taken together, we provide new details on the mechanism of this type of sRNA mediated regulation.

In manuscript II we describe in detail how to perform site-directed mutagenesis. Site-directed mutagenesis was an important tool for determining the mechanism described in manuscript I. We hope that the manuscript will assist other research groups in performing similar experiments to investigate mechanistic details of other regulatory system.

In manuscript III we characterized the overall regulatory circuit and biological function of a two-component system involved in virulence. We find that it responds to glycan structures which are also present on host cells. Upon stimulation by these structures, the system induces expression of genes required for sequential degradation, transport and catabolism of glycans. We find its role in virulence is not dependent on adhesion or invasion, and instead suggest that it is required for nutrient acquisition in niches devoid of readily available carbohydrates. Furthermore, the system and its regulon are specifically conserved in related species that are capable of catabolizing glycans. Thus, we provide new insight into a regulatory circuit conserved in several pathogenic species.