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The timing of streptolysin O release is controlled by the *slo*R operon

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Abstract. Streptolysin O (SLO) is a member of the thiol-activated cytolysins found in a number of Gram positive pathogenic bacteria. Often accompanying these cytolysins is a second gene first identified in *Clostridium perfringens* as the putative perfringolysin O regulator (pfoR). Members of the pfoR family are integral membrane proteins and exclusively found in exotoxin producing low %G+C Gram positive bacteria. The homolog of pfoR found in *Streptococcus pyogenes* is gene Spy0146 or *slo*R, a member of a three gene operon. In contrast to all other species, *slo*R is co-transcribed with another gene, Spy0145, a member of a large family of predicted transcriptional regulators of the *yjg*F family. The third gene (Spy0144) is a predicted DNA binding protein that may function as a regulator for the operon. We previously had shown that inactivation of SloR modulates the expression of SLO expression. To examine the effect of the *slo*R operon on SLO hemolysis, *S. pyogenes* strain NZ131 or its isogenic mutants for *slo*R (OK85) or Spy0144 (NZ131ΔSpy144) were grown in rich medium, with supernatant samples harvested between early-logarithmic and stationary phases. Trypan blue was used to inhibit SLS activity. Hemolysis in strain OK85 (*slo*R-) was delayed as compared to wild type by approximately one cell division, while strain NZ131ΔSpy0144 showed an early expression of hemolysis as compared to wild type. The inactivation of Spy0144 appears to be related to increased expression of *slo*R and up-regulation of SLO. Restoration of a functional copy of Spy0144 on a bacteriophage integration vector was able to partially restore the wild type phenotype. The findings suggest that the *slo*R operon may encode an environmental sensor that relays some external signal to the cell, modulating the transcription of SLO through a yet to be determined molecular pathway. © 2005 Published by Elsevier B.V.

Keywords: Streptococcus pyogenes; Group A streptococcus; Streptolysin O; Cholesterol dependent cytolysin

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1. Introduction

Previously, we identified a group A streptococcus (GAS) gene, *slo*R (Spy0146), that modulates expression of the cholesterol dependent cytolysin streptolysin O (SLO; *slo*) [1]. The SloR protein, named after the previously identified perfringolysin O regulator from *Clostridium perfringens* [2], is a predicted integral membrane protein with 9 probable membrane spanning domains. Homologs of *slo*R are only found in a limited group of Gram positive species that are notable exotoxin producers (e.g., *Bacillus anthracis*, *Clostridium botulinum*, *Clostridium tetani*, and *C. perfringens*) and are often found positioned next to the toxin gene on the chromosome 2).

The gene immediately upstream of *slo*R, gene Spy0145 (*yjg*F homolog), is co-transcribed on a bicistronic message with *slo*R (unpublished results). Spy0145 is related to a large number of proteins from a wide range of bacteria and eukaryotes, and while its specific function is unclear, studies in other species suggest that it is important in the regulation of metabolism. In no other species for which sequence data is available have the homologs of these two genes (*yjg*F and *slo*R) been linked together as a coordinated genetic unit, and therefore, the bicistronic pairing of these genes appears to be unique to GAS. Further, a third gene (Spy0144), encoding a conserved hypothetical protein with a predicted helix–turn–helix motif, is positioned upstream of this operon on the opposite coding strand such that its promoter overlaps the promoter controlling *yjg*F and *slo*R. The overall organization of these genes resembles that of many operons under control of a repressor/regulator (Fig. 1). Here we show that inactivation of this putative regulatory gene, Spy0144, changes the timing of SLO release, causing a premature release of the toxin as compared to the wild type strain.

2. Methods

NZ131 is an M49 GAS strain that was originally isolated from a patient with nephritis and whose genome has been completely sequenced (in preparation). Gene names follow the convention of the M1 genome (GenBank accession AE004092). The construction of OK85, an isogenic derivative of NZ131 inactivated for *slo*R, has been previously described [1]. Strain NZ131ΔSpy0144 has a Spy0144 gene knockout that was created by electroporation of NZ131 with a streptococcal suicide plasmid specifying erythromycin resistance and

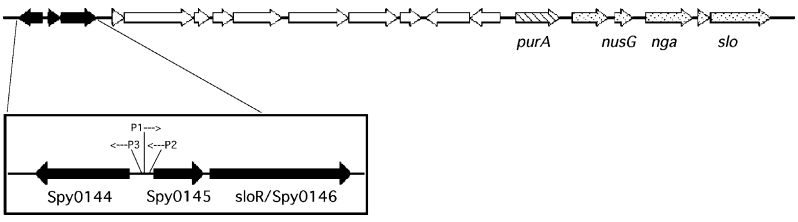

Fig. 1. The streptolysin O region of the *S. pyogenes* genome is shown from Spy0144 through *slo*. The genes preceding *slo*, *nga*, *nus*G (a transcription factor), and *pur*A, are identified. The enlargement shows a detailed map of the *slo*R (Spy0146) region. Promoter analysis suggested that three probable promoters are found between Spy0144 and Spy0145, one (P1) promoting transcription of Spy0145 and Spy0146 while the other two (P2 and P3) potentially promoting Spy0144 (a conserved hypothetical protein with a helix–turn–helix motif).