Function and control of the ssg genes in streptomyces

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Chapter I

Introduction

Streptomycetes are Gram-positive soil-dwelling bacteria, in appearance similar to filamentous fungi. In fact, *Streptomyces* owes its name to this apparent likeness, when in 1943 Waksman and Henrici combined the orginal names given to the first actinomycetes (Waksman and Henrici, 1943), "Actinomyces" (Greek for "ray fungus") and "Streptothrix" (Greek for "twisted hair"). *Streptomyces* genomes are larger than those of most other bacteria; with around 7950 predicted genes (7847 encoding proteins, 18 rRNAs, 65 tRNAs and several small RNAs), the genome of *Streptomyces coelicolor* encodes almost twice as many genes as well-known bacteria such as *Escherichia coli*, *Bacillus subtilis* and *Mycobacterium tuberculosis* (all have approximately 4000 predicted genes), and carries even more genes than the lower eukaryote *Saccharomyces cerevisiae* (6203 predicted genes) (Bentley *et al.*, 2002). This large genome size reflects the complex morphogenesis and saprophytic nature of streptomycetes, which is highlighted by the presence of no less than 65 sigma factors, a wealth of polysaccharide hydrolases and over 200 ABC transporters; this allows the organism to rapidly respond to environmental changes and utilise many complex carbon sources (*e.g.* chitin, cellulose, lignin, mannan, xylan and agar).

During its life cycle, *Streptomyces* undergoes two apparently different events of cell division. Initially, the cell division machinery produces irregularly spaced septa ("crosswalls") in the vegetative hyphae that delimit the multi-nucleoid hyphal cells. During the reproductive phase, up to a hundred septa are simultaneously formed in the sporogenic aerial hyphae, eventually resulting in the production of mono-nucleoid spores (Chater, 2001; Flärdh and van Wezel, 2003). In contrast to septation in vegetative hyphae, developmental cell division results in physical separation of the spores. Instead of the canonical cell division control proteins, most of which were identified during studies on *E. coli* and *B. subtilis*, several unique protein families have been identified that play a role in the control of cell division in streptomycetes (Chater and Chandra, 2006). This thesis deals with one such family, the SsgA-like proteins (SALPs). Since its discovery during the mid-90s, SsgA (for sporulation of *Streptomyces griseus*) has been shown to play an important role in the activation of sporulation-specific cell division in *Streptomyces* on solid surface and in liquid medium. We now know streptomycetes generally have six to eight SALP homologues, all of which play an important and unique role in the control of sporulation (Noens *et al.*, 2005). In

Chapter II, 10 years of research on the SsgA-like proteins in actinomycetes is reviewed.

This work started at a time when further insight into the regulation of *ssgA* was necessary to better understand its role during development and to place this important morphogen in the life cycle of *Streptomyces*. The transcriptional regulation of *ssgA* in the model organism *Streptomyces coelicolor* was extensively studied in different genetic backgrounds, results of which are presented in Chapter III. These indicated that transcription of *ssgA* directly depends on the DNA-binding regulator SsgR, but is independent of other essential sporulation genes known at that time. Major differences were observed compared to the regulation of the *ssgA* orthologue in *S. griseus* (Yamazaki *et al.*, 2003), which could be correlated to the ability of *S. griseus* to produce spores in submerged culture, a property that *S. coelicolor* lacks. In Chapter IV, the survey of transcriptional regulation is expanded so as to include the analysis of the six *S. coelicolor ssgA*-like genes (*ssgB*-*G*). Results indicate that all but *ssgD* are developmentally controlled and repressed by glucose. During the course of development, *ssgB* transcript levels were found to be strongly dependent on two sporulation genes (*i.e. whiA* and *whiH*). We furthermore obtained and compared *ssgA* sequences from a large number of streptomycetes. In addition to the role of transcriptional regulation of *ssgA*, phylogenetic evidence suggested that there is a relationship between particular amino acid residues and the ability of some strains to produce spores in submerged culture. In order to obtain more insight into the role of individual amino acid residues in its function, SsgA was subjected to an extensive mutational study by setting up a novel method to create, maintain and screen a library of random mutants (see Chapter V).

At the start of this work, SALPs had only been identified in streptomycetes, but with the publication of a great number of full genome sequences of various actinomycetes in recent years, genes encoding SALPs have been found in a number of morphologically diverse actinomycetes. In Chapter VI, genetic evidence is presented that strongly suggests that SsgB is the archetype of the SALP family. Results indicate that SsgB plays an important role in septum-site localization during developmental cell division and that this function is conserved even in distantly-related actinomycetes.

Finally, the results presented in this thesis are summarised in Chapter VII, and the implications with respect to actinomycete morphogenesis are discussed.