Function and control of the ssg genes in streptomyces

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Chapter VII

Summary and discussion

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Nederlandse samenvatting

Streptomycetes are filamentous bacteria belonging to the order *Actinomycetales*, members of which most likely share their last common ancestor approximately one billion years ago (Embley and Stackebrandt, 1994). Actinomycetes come in a wide variety of morphologies, such as coccoid (*e.g. Micrococcus*), rod-shaped (*e.g. Mycobacterium*) or mycelium-forming (*e.g. Streptomyces*). SsgA-like proteins (SALPs) each play a distinctive and important role in the control of morphogenesis in streptomycetes (Noens *et al.*, 2005). Unique to actinomycetes, SALPs have thus far been identified in a limited number of actinomycete-genera from various taxonomic suborders. Interestingly, a clear correlation exists between the complexity of the morphology and the number of SALPs found in actinomycetes, with one SALP protein occuring in actinomycetes that produce single or no spores and multiple SALPs in actinomycetes that produce an aerial mycelium and spore chains or sporangia (Chapter II). In this thesis, the regulation of SALPs and their function in morphological differentiation of actinomycetes, in particular of *Streptomyces*, are adressed.

SsgA and the control of *Streptomyces* morphogenesis

The transcriptional regulation of *ssgA* in the model organism *Streptomyces coelicolor* grown on solid-surface was extensively studied (Chapter III), and revealed major differences compared to the regulation of its orthologue in *Streptomyces griseus* (Yamazaki *et al.*, 2003). Life cycle-dependent transcription of *ssgA* in *S. coelicolor* and *S. griseus* originates from two transcriptional start sites. While one of these promoters is essentially the same in both species for both sequence and location, the second one is markedly different (Chapter III). Sequence homology strongly suggests that this conserved promoter is common to all *Streptomyces* species (Chapter IV). In *S. coelicolor*, both *ssgA* transcripts are *trans*-activated by and dependent on SsgR. SsgR binds to a DNA fragment upstream of *ssgA in vitro*, and most likely to a 33 nucleotides A/T-rich sequence surrounding the stop codon of *ssgR* (Chapter III). This sequence is highly conserved in 18 different streptomycetes (Chapter IV). In contrast, *ssgA* transcription in *S. griseus* is dependent on the A-factor-pathway-controlled AdpA (Horinouchi and Beppu, 1994; Ohnishi *et al.*, 2005), while overall transcription is less strongly affected by the cognate SsgR (named SsfR in this species). Interestingly, mutation of the *ssgR* orthologue in this species strongly reduced

transcription originating from the conserved transcriptional start site of *ssgA* (Yamazaki *et al.*, 2003). Considering the sequence conservation of the common promoter and the putative SsgR binding site, and the SsgR-dependence of the common promoter in *S. coelicolor* and *S. griseus*, we anticipate that this promoter may be controlled by SsgR in perhaps all *Streptomyces* species. SsgA is an important determinant for sporulation of *S. griseus* in submerged culture (Kawamoto and Ensign, 1995a; Kawamoto *et al.*, 1997). The additional transcriptional activation by AdpA in *S. griseus* is at least one explanation why this species is able to sporulate in submerged culture, while *S. coelicolor* is not. In line with this, there is no detectable transcription of *ssgA* in submerged cultures of *S. coelicolor*, while it is strongly expressed in *S. griseus* (Kawamoto *et al.*, 1997; van Wezel *et al.*, 2000a; van Wezel *et al.*, 2000b). In liquid-grown cultures of wild-type *S. griseus*, *ssgA* transcription is induced upon nutritional depletion, a condition known to induce submerged sporulation. However, transcript levels of *adpA* and *ssfR* are not enhanced in this manner (Chapter IV). This suggests that *ssgA* transcription is repressed in complex liquid media, and induced upon nutritional shift-down. The hyper-sporulating *S. griseus* strain SY1 produces SsgA at high levels in complex liquid media (Kawamoto *et al.*, 1997; van Wezel *et al.*, 2000a). *ssgA* transcript levels were no longer upregulated by nutritional shift-down in this strain. The mutation causing enhanced SsgA protein levels in complex medium is still unknown, and elucidating this should provide important information as to how *ssgA* responds to the nutritional state of the environment.

Excitingly, phylogenetic analysis of the *ssgA* gene products from 18 streptomycete species revealed a clear correlation between the ability of particular streptomycetes to produce submerged spores and their cognate SsgA proteins (Chapter IV). This strongly suggests that, in addition to the role of transcriptional control, particular amino acid (aa) residues play a role in the ability of SsgA to induce spore formation in liquid-grown cultures. SsgA has been suggested to stimulate septation by modifying the cell wall at specific locations (Noens *et al.*, 2007). Overexpression of *S. griseus* SsgA strongly increases the degree of fragmentation in liquid-grown mycelium (van Wezel *et al.*, 2000a), which is directly proportional to the frequency of septation (van Wezel *et al.*, 2006). As discussed in Chapter II, this mycelium is also much more sensitive to

heat, osmotic stress (*e.g.* high sucrose concentrations) and SDS-treatment than the parental strain, suggesting that SsgA overexpression results in a weakened cell wall. Interestingly, similar overexpression of *S. coelicolor* SsgA is far less effective (Gilles van Wezel, unpublished data). This highlights a major discrepancy between the functions of *S. coelicolor* SsgA and *S. griseus* SsgA, relating to their ability to induce septation in liquid-grown mycelium. This prompted the development of a method to efficiently create, maintain and screen a library of random *S. coelicolor ssgA* variants, in order to study the role of specific aa residues in the function of SsgA (Chapter V). The residues essential for solid-surface sporulation were found clustered in three main sections of SsgA. The majority of these residues is conserved among all SALPs. However, three essential residues (*i.e.* L29, D58, and S89) were conserved only in SsgA, suggesting these are involved in an SsgA-specific function. The implications of these results and the use of this library with regards to the ability to induce submerged sporulation are currently under investigation. Most of the important aa residues highlighted by the mutational analysis are located in the buried hydrophobic core of the structure of *S. coelicolor* SsgA (Appendix B), which was modelled to the available crystal structure of SsgB from *Thermobifida fusca*. As discussed in Chapter II, the recently elucidated structure of SsgB from *Thermobifida fusca* revealed structural similarity to a class of ssDNA/RNA-binding proteins. If indeed SALPs interact with RNA (or ssDNA), one possibility is that their activity is modulated by interaction with such molecules. Bacterial genomes generally encode several non-coding RNA's (ncRNA) which control a variety of cellular processes (Vogel and Sharma, 2005), including modulating protein activity (Wassarman and Storz, 2000). A recent study highlighted several previously unpredicted ncRNA's in *Streptomyces* (Panek *et al.*, 2008). This possibility requires investigation.

Expression of SALPs during early and late developmental checkpoints

The decision to enter the developmental phase of the life cycle is irreversible, and the formation of aerial hyphae and spores is an energy-consuming process. The onset of development is therefore tightly controlled in a nutrient-dependent manner (Chater and Losick, 1997). This is for example illustrated by the negative effect glucose and other type I carbon sources have on sporulation (Kwakman

and Postma, 1994). The developmental SALP-encoding (*ssg*) genes (*i.e. ssgABCEFG*) are all subjected to carbon catabolite repression (Chapter IV), providing novel insight into how nutrient availability controls the later stages of development (*i.e.* sporulation). In contrast, *ssgD* is expressed at a high level already during the earliest stages of growth, and its expression is not affected by glucose. This strongly suggests a role for *ssgD* during vegetative growth. However, mutation of *ssgD* only noticeably affected the integrity of the cell wall in aerial hyphae and spores, and under the conditions tested no clear defects were observed in vegetative hyphae (Noens *et al.*, 2005).

On solid-surface, colony differentiation is controlled by several important sporulation genes, which include six genes essential for the development of aerial hyphae up to the point of septum formation, namely: *whiA*, *whiB*, *whiG*, *whiH*, *whiI* and *whiJ*, all encoding transcription factors (Chater, 1972; Chater and Chandra, 2006). *ssgRA* are transcribed independently of these six early *whi* genes, while *ssgB* transcript levels were strongly down-regulated in *whiA* mutants, and abolished in *whiH* mutants (Chapter III and IV). Of all *S. coelicolor* SALP null mutants, *ssgA* and *ssgB* mutants have a non-sporulating (*whi*) phenotype. *ssgB* mutants have a strict *whi* phenotype on all media, and produce large white colonies (Keijser *et al.*, 2003; Sevcikova and Kormanec, 2003). The absence of *ssgB* transcription may therefore provide a structural explanation for the sporulation-deficient phenotype of *whiH* mutants. This possibility is currently under investigation. On the other hand, *ssgA* mutants have a conditional *whi* phenotype, capable of producing spores on mannitol-containing media, but not in the presence of glucose (Jiang and Kendrick, 2000b; van Wezel *et al.*, 2000a). Such a conditional phenotype is rare among *whi* mutants. This, and the observation that its expression is *whi*-independent, strongly suggest that SsgA provides an alternative to solid-surface sporulation, underlining its important role in submerged sporulation (see above).

SsgB plays a crucial role in septation

Clear similarities were observed in the genetic locus of *ssg* genes in all SALP-containing actinomycetes to that of *ssgB* in *Streptomyces*. This led to the proposition that *ssgB* is the archetype of the SALP family, and that other *ssg* genes have been derived from spread and/or duplication of *ssgB* (Chapter VI).

The presence of several tRNA genes in the vicinity of *ssgB* in all actinomycetes suggests that *ssgB* was originally acquired through horizontal gene transfer, as tRNA loci are implicated as common sites for the integration of foreign sequences (Ochman *et al.*, 2000). *Streptomyces ssgB* mutants produce smooth aseptate aerial hyphae and are deficient in sporulation (Keijser *et al.*, 2003; Sevcikova and Kormanec, 2003). Plasmid-borne *ssgB* orthologues from *Acidothermus cellulolyticus*, *Kineococcus radiotolerans*, *Saccharopolyspora erythraea* and *Salinispora tropica* to some degree all restored sporulation-specific cell division in aerial hyphae of the *ssgB* mutant, indicating that SsgB has a universally conserved function in actinomycete morphogenesis. However, several defects were observed in sporulation in the complemented strains, including abnormal septal spacing and highly variable spore sizes. In all bacteria the division ring consists of polymeric rings of FtsZ molecules (the Z-ring). Interestingly, *ssgB* and the developmental promoter of *ftsZ* are both dependent on the sporulation sigma factor gene *whiH* (Chapter IV; (Flärdh *et al.*, 2000)), resulting in simultaneous up-regulation of both genes in sporogenic aerial hyphae. Moreover, co-expression studies of fluorescent protein fusions indicate that SsgB is recruited prior to Z-ring formation in aerial hyphae (Joost Willemse and Gilles van Wezel, unpublished data). The possibility that SsgB affects septum-site localization through direct interaction with FtsZ is currently under investigation (see "Future research" section). If no direct interaction with FtsZ is found, considering the universally conserved function of SsgB, any putative interacting partner is also likely to be conserved in the genomes of all SALP-containing actinomycetes. The genome sequences of *Acidothermus cellulolyticus* and *Thermobifida fusca* can prove very useful on the search for or elimination of such candidate partners, especially because of their considerably smaller sizes (around 2.4 and 3.6 Mbp, respectively), which allows searching for the proverbial needle in a considerably smaller haystack.

SALPs and the evolution of actinomycete morphogenesis

Functional SsgB orthologues occur in morphologically very distinct actinomycetes (Chapter VI). Multiple SALPs occur in actinomycetes that produce aerial hyphae and complex multisporous structures, namely in the streptomycetes (spore chains), in *Frankia* species (sporangia) and in *Saccharopolyspora erythraea*

(short spore chains) (Chapter II). Functional analysis in streptomycetes and phylogenetic evidence strongly suggests that SALPs are crucial for (spore) septum formation in all SALP-containing actinomycetes, that at least two SALPs are required to produce more than one spore septum simultaneously, and that multiple (three or more) SALPs are required to coordinate the production of long spore chains or sporangia.

Where do SALPs fit in actinomycete evolution? SsgB occurs in all sequenced genomes belonging to different taxonomic families within the suborder *Frankineae* (*i.e. Acidothermus*, *Frankia* and *Kineococcus*), suggesting that *ssgB* arose early on in the evolution of this taxon. Genetic evidence further suggests that original acquisition occurred through horizontal gene transfer, providing a plausible explanation for the complete absence of SALP homologues from a considerable number of other fully sequenced actinomycete genomes (*e.g.* Corynebacterium, Mycobacterium). Multiple SALPs occur exclusively in aerial mycelium-forming actinomycetes, but it is unclear whether this morphological trait has been derived from a common origin. Whole genome comparisons of the *Frankia* and *Streptomyces* revealed a great deal of similarity, as if to suggest their mycelial morphology had a common origin, eventhough a phylogenetic tree of 16S rRNA implied an ancient common ancestry (Ventura *et al.*, 2007). Conversely, whole genome comparison revealed that *Saccharopolyspora erythraea*, although formerly identified as *Streptomyces erythreus*, is only distantly related to *S. coelicolor* (Oliynyk *et al.*, 2007). Nevertheless, multiple (two or more) SALPs occur exclusively in actinomycetes which produce multisporous structures, suggesting that the acquisition of additional SALPs, through spread and/or gene duplication, occurred relatively recently in actinomycete evolution.

Future research

As a logical extension to the work presented in this thesis, a number of research lines can be considered. First, the role of SsgA during submerged sporulation should be investigated further. A great deal is now known about the transcriptional regulation of *ssgA*, but it is unclear how *ssgA* transcription is induced in liquid-grown cultures of *S. griseus* by nutritional shift-down. Uncovering the mutation resulting in enhanced expression of SsgA in the hyper-

sporulating strain SY1 will undoubtedly provide valuable insight into this question. In light of recent observations, the relationship between particular aa residues of SsgA and its ability to induce submerged sporulation should be investigated. This will improve our understanding as to how SsgA stimulates (submerged) sporulation. The random mutant library described in Chapter V, can prove valuable for this purpose.

Second, multiple SALPs occur exclusively in actinomycetes that produce spore chains or sporangia, and as suggested above, these are perhaps involved in coordinating the formation of multiple septa and/or spores. Single mutants of *ssgC-G* still produce spore chains, although several defects were observed in septum-site localization, DNA segregation/condensation, spore-wall synthesis and autolytic spore separation (Noens *et al.*, 2005). Combinations of SALP mutants in for example *S. coelicolor* may result in streptomycetes producing single spores or short spore chains. Conversely, it would be very interesting to see if multiple SALPs can trigger the production of multisporous structures in actinomycetes that normally produce few or no spores. For such experiments *Saccharopolyspora* is a good candidate as the basic machinery to produce short spore chains is present. The single-spore forming *Micromonospora* could be used in a similar fashion, to test if the expression of SALPs from *S. coelicolor* could lead to spore-chain formation.

Finally, a number of important questions regarding the mode-of-action of SALPs need to be adressed in the future in order to enhance our understanding of this still rather mysterious protein family. The overall sequence similarity between SALP homologues is highly suggestive of an essential common aspect in their specialized functions. Live-cell imaging (*e.g.* FRET-FLIM) and biochemical methods (two-hybrid screening) should elucidate the SALP interaction partners. In view of evidence presented in Chapter VI, a possible direct interaction of SsgB with FtsZ is currently under investigation. Another question is, how are SALPs themselves recruited? SsgA and SsgB are most likely recruited prior to Z-ring formation (Joost Willemse and Gilles van Wezel, unpublished data), and hence their localization is by definition not dependent on the divisome. The dynamic and organized localization of SsgA in aerial hyphae (Noens *et al.*, 2007) is suggestive of a relationship with the symmetrical structure of the cell (*i.e.* the *Streptomyces* cytoskeleton).

Nederlandse samenvatting

Streptomyces is een Grampositieve grondbacterie behorend tot de orde van de actinomyceten, waarvan de laatste gemeenschappelijke voorouder waarschijnlijk één miljard jaar geleden leefde (Embley and Stackebrandt, 1994). In de eerste fase van de levenscyclus, produceert *Streptomyces* een netwerk van vertakkende hyfen, het zogenaamde vegetatieve mycelium. Wanneer de omstandigheden ongunstig worden, *e.g.* bij een te kort aan voedingsstoffen, ondergaan kolonies een morfologische ontwikkeling, waarbij luchthyfen worden gevormd op het vegetatieve myceliumnetwerk. Deze luchthyfen worden door tientallen septa verdeeld in ketens van mononucleoide sporen, welke uiteindelijk fysiek worden gescheiden. SsgA-achtige eiwitten (SALPs) zijn eiwitten die uniek voorkomen in actinomyceten en elk een belangrijke rol spelen bij de controle van de sporulatie in *Streptomyces* (Noens *et al.*, 2005). Het onderzoek zoals dat in dit proefschrift beschreven staat, was gericht op de rol van de (gen)expressie en de functie van deze relatief onbekende familie van eiwitten bij de morfologische ontwikkeling.

SsgA en de controle van *Streptomyces* morfogenese

SsgA stimuleert septumvorming in *Streptomyces* hyfen en bepaalt daarmee wanneer sporulatie op vaste voedingsbodems en in vloeibare culturen plaatsvindt (Jiang and Kendrick, 2000b; Kawamoto and Ensign, 1995a; Kawamoto *et al.*, 1997; van Wezel *et al.*, 2000a). Transcriptie-analyse van *ssgA* tijdens de groei van de modelstreptomyceet *S. coelicolor* op vaste voedingsbodems toonde aan dat de groeifase-afhankelijk transcriptie van *ssgA* door twee promotoren wordt verzorgd en dat de transcriptie geheel afhankelijk is van de DNA-bindende regulator SsgR (hoofdstuk III). De sequenties van de meest waarschijnlijke bindingsplek van SsgR en één van de twee promotoren (p2 in *S. coelicolor*) vertonen sterke overeenkomsten in alle onderzochte streptomyceten (hoofdstuk IV). Het is daarom aannemelijk dat SsgR de transcriptie van *ssgA* tot op zekere hoogte activeert of stimuleert in alle streptomyceten. In *Streptomyces griseus* daarentegen, wordt transcriptie van *ssgA* geactiveerd door de A-factor-afhankelijke regulator AdpA (Horinouchi and Beppu, 1994; Ohnishi *et al.*, 2005) en speelt de ortholoog van SsgR in deze soort een minder belangrijke rol van betekenis (Yamazaki *et al.*, 2003). In vloeibare culturen van *S. coelicolor* is er

geen waarneembare transcriptie van *ssgA*, terwijl deze sterk tot expressie komt in *S. griseus* (Kawamoto *et al.*, 1997; van Wezel *et al.*, 2000a). De verschillende regulatie van *ssgA* geeft daarom tenminste één verklaring waarom *S. griseus* in staat is tot sporulatie in vloeibare culturen, terwijl *S. coelicolor* dit niet is. In vloeibare culturen van *S. griseus* wordt transcriptie *ssgA* sterk gestimuleerd bij de overgang van complex naar minimaal medium (*nutritional shift-down*), een conditie waarbij ook sporulatie geïnduceerd wordt. De transcriptieniveau's van *adpA* en *ssgR* blijven daarentegen hierbij onveranderd (hoofdstuk IV). De hypersporulerende *S. griseus* mutant SY1 produceert aanzienlijk meer SsgA eiwit in complexe media (Kawamoto *et al.*, 1997; van Wezel *et al.*, 2000a). *S. griseus* SY1 bevat geen mutaties in de genen voor *ssgA, ssgR* en die voor de A-factor afhankelijke cascade *afsA*, *arpA* en *adpA*. Dit alles wijst op een nog onbekend controle mechanisme van *ssgA* expressie in complex vloeibare media. Het identificeren van de mutatie in SY1 zal belangrijke informatie opleveren over deze regulatie.

Overexpressie van *S. griseus* SsgA induceert fragmentatie van mycelium in vloeibare culturen (van Wezel *et al.*, 2000a). Verder is dit mycelium gevoeliger voor hitte, osmotische stress en behandeling met natriumdodecylsulfaat (SDS) dan de oorspronkelijke stam, hetgeen suggereert dat overexpressie van SsgA resulteert in een verzwakte celwand (besproken in hoofdstuk II). Vergelijkbare overexpressie van *S. coelicolor* SsgA daarentegen is aanzienlijk minder effectief (Gilles van Wezel, niet-gepubliceerde data). Dit duidt op een groot verschil in functie van SsgA in deze twee soorten in vloeibare culturen. Fylogenetische analyse van de *ssgA* genproducten van 18 streptomyceten onthulde een duidelijke correlatie tussen de mogelijkheid van bepaalde stammen tot sporenvorming in vloeibare media en de aminozuursequentie van SsgA (hoofdstuk IV). Om meer inzicht te krijgen in de rol van specifieke aminozuurresiduen in de functie van SsgA is een methode ontwikkeld waarmee een verzameling van 1500 gemuteerde SsgA-varianten is gemaakt. Hierbij zijn specifieke mutaties opgehangen aan de activiteit van het eiwit (hoofdstuk V). De aminozuurresiduen die van belang zijn voor sporulatie op vaste voedingsbodems zijn geclusterd in drie gedeeltes van het eiwit. Het overgrote deel van deze residuen is geconserveerd binnen alle SALP eiwitten, terwijl drie residuen (*i.e.* L29, D58, and S89) alleen in SsgA geconserveerd zijn. Deze laatste zijn

waarschijnlijk betrokken bij een SsgA-specifieke functie. De verdere implicaties hiervan met betrekking tot de rol van SsgA bij de sporulatie in vloeibare media worden op dit moment onderzocht. De krystalstructuur van de SsgB ortholoog van *Thermobifida fusca* (op basis van de resultaten in hoofdstuk V), werd tijdens het schrijven van dit proefschrift opgelost en openbaar gemaakt door het *Joint Center for Structural Genomics* (besproken in hoofdstukken II en VII). Dit onthulde verrassende structurele overeenkomsten met een klasse van ssDNA/RNA-bindende eiwitten. Het combineren van deze nieuwe driedimensionale gegevens met de mutatie-analyse van SsgA zal ongetwijfeld veel belangrijke informatie opleveren over de relatie tussen structuur en functie.

SALP-expressie in *S. coelicolor* tijdens de levenscyclus

Morfologische ontwikkeling is een energieconsumerend proces en de beslissing om hiertoe over te gaan is voor *Streptomyces* onomkeerbaar. De overgang naar de ontwikkelingsfase wordt daarom streng gecontroleerd (Chater and Losick, 1997). Dit wordt geïllustreerd door het remmende effect van glucose en andere type I koolstofbronnen op de sporulatie (Kwakman and Postma, 1994). Expressie van de groeifase-afhankelijke *ssg* genen (*i.e. ssgABCEFG*) wordt onderdrukt door glucose. Daarentegen komt *ssgD* hoog tot expressie voor het begin van de ontwikkelingsfase en wordt zijn expressie niet onderdrukt door glucose (hoofdstuk IV). Dit suggereert dat SsgD ook een rol speelt tijdens de vegetatieve groei. Mutatie van *ssgD* tastte echter ogenschijnlijk alleen de celwand van luchthyfen en sporen aan (Noens *et al.*, 2005).

Tenminste zes sporulatie- of *whi* ("white") genen zijn essentieel voor de ontwikkeling van luchthyfen op vaste voedingsbodems tot aan de vorming van septa, namelijk *whiA*, *whiB*, *whiG*, *whiH*, *whiI* and *whiJ*, allen coderend voor transcriptiefactoren (Chater, 1972; Chater and Chandra, 2006). Transcriptie-analyse van de *ssg* genen in deze zes essentiële *whi*-genen, toonde aan dat *ssgRA* onafhankelijk worden getranscribeerd van deze genen (hoofdstuk III). Transcriptie van *ssgB* was sterk gereduceerd in een *whiA* mutant en vond niet plaats in een *whiH* mutant (hoofdstuk IV). *ssgB* mutanten hebben een klassiek niet-sporulerend fenotype en produceren geen septa in luchthyfen (Keijser *et al.*, 2003; Sevcikova and Kormanec, 2003). De afwezigheid van *ssgB* transcripten is een mogelijke verklaringen voor het gebrek aan sporulatie in *whiH* mutanten.

Aan de andere kant vertonen *ssgA* mutanten een voorwaardelijk "white" fenotype en zijn ze in staat sporen te vormen op mannitol-bevattend medium, maar niet in de aanwezigheid van glucose (Jiang and Kendrick, 2000b; van Wezel *et al.*, 2000a). Tezamen met het feit dat *ssgA* transcriptie "*whi*-onafhankelijk" is, suggereert dit dat SsgA een alternatieve route naar sporulatie mogelijk maakt, hetgeen zijn essentiele rol in sporulatie in vloeibare media onderstreept.

De geconserveerde functie van SsgB in actinomyceten

ssgB is de meest waarschijnlijke archetype van de SALP familie en orthologen komen voor in alle SALP-bevattende actinomyceten (hoofdstuk VI). De aanwezigheid van een aantal tRNA genen in de loci van alle *ssgB* orthologen, suggereert dat *ssgB* origineel verkregen is door horizontale genoverdracht, daar tRNA loci preferentieel worden gebruikt voor de integratie van vreemd DNA (Ochman *et al.*, 2000). Andere *ssg* genen zijn waarschijnlijk ontstaan door verspreiding en/of duplicatie van *ssgB*. De *ssgB* genen van vier ver verwante actinomyceten (*i.e. Acidothermus cellulolyticus*, *Kineococcus radiotolerans*, *Saccharopolyspora erythraea* en *Salinispora tropica*) waren in staat de sporulatie-specifieke celdeling in luchthyfen van de *ssgB* mutant van *S. coelicolor* te herstellen (hoofdstuk VI). Dit geeft aan dat SsgB een universeel geconserveerde functie heeft bij de morfogenese van actinomyceten. Een aantal imperfecties werden waargenomen in het sporulatieproces in aanwezigheid van deze vreemde SsgB orthologen, namelijk: een verzwakte sporenwand, een afwijkende septumafstand en een variabele sporengrootte. De afwijkende afstand tussen de septa impliceert dat SsgB tijdens sporulatie betrokken is bij de localisatie van celdelingssepta. In alle bacteriën is de eerste stap in septatie de localisatie van polymeerringen van FtsZ (Z-ringen), de bacteriële homoloog van tubuline. Interessant genoeg zijn transcriptie van *ssgB* en de sporulatiepromoter van *ftsZ* beide afhankelijk van het sporulatiegen *whiH* (hoofdstuk IV; (Flärdh *et al.*, 2000)). Verder wordt SsgB vóór de formatie van Z-ringen gerecruteerd (Joost Willemse en Gilles van Wezel, niet-gepubliceerde data). De relatie tussen SsgB en FtsZ wordt op dit moment in meer detail onderzocht.